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The Adult Consequences of Pediatric Endocrine Disease, II: Turner Syndrome

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INTRODUCTION

In Europe, the syndrome we know as Turner syndrome (TS) is known as Ullrich syndrome. This is because Ullrich described the syndrome in 1930, 8 years before Henry Turner described the entity in the United States. The patients were similar, but their ages were not. Therefore, the signs and characteristics differed. Ullrich described a group of *preadolescent* female children with proportionate short stature and various congenital anomalies, including pterygium colli and a wide carrying angle at the elbow, among others. Turner described *adolescent* girls with the same phenotype who remained sexually undeveloped.

In the late1950s, when chromosomal karyotyping became available, the etiology was clarified. TS was demonstrated to be a genetic disease resulting from the complete or partial absence of 1 of the sex chromosomes. The initially described patients had a total of 45 chromosomes and only one X chromosome. Subsequently, patients were identified who exhibited mosaicism (for example, 45,X/46,XX karyotypes). Others had a partial deletion of 1 of the arms of the second X chromosome, usually with duplication of the remaining arm (p or q), and a resultant second X chromosome with a double gene dose of the remaining arm and zero dose of the absent arm. TS patients with the so-called isochromosome form are designated karyotypically as 46,X,I(Xq), as usually the short arm is absent and the long arm is duplicated. Partial deletions also occur in some patients on the tips of or part of the p and q arms of 1 of the X chromosomes. Sometimes the sticky remaining end portions adhere, so that these chromosomes are shaped like a ring. The karyotype designation of patients with this gene phenotype is 46,X,r(X). Less frequently there are other associated chromosome and gene anomalies, including the presence of a partial Y chromosome as the second chromosome component.[1]

Considering the types and extent of chromosome and gene anomalies with the association of various somatic anomalies and diseases is important. For example, TS individuals with the 45,X karyotype usually have some or all of the congenital somatic anomalies associated with the syndrome, while many of those with mosaicism, isochromosomes, partial deletions, or ring X chromosomes have fewer somatic anomalies apart from short stature and sexual infantilism. The pertinence of this concept of genetic and somatic association will be more evident as we consider the adult consequences of this pediatric endocrine disease.

With this orientation, a discussion of the *mortality* and *morbidity* of TS patients in adulthood is presented. The object in this presentation is to (1) help physicians caring for adult TS patients understand the need to work closely with these patients to prevent, diagnose, and treat adverse consequences; and (2) help the pediatrician in facilitating the transfer of TS patients to internists, gynecologists, family practitioners, and other appropriate physicians.

In This Issue

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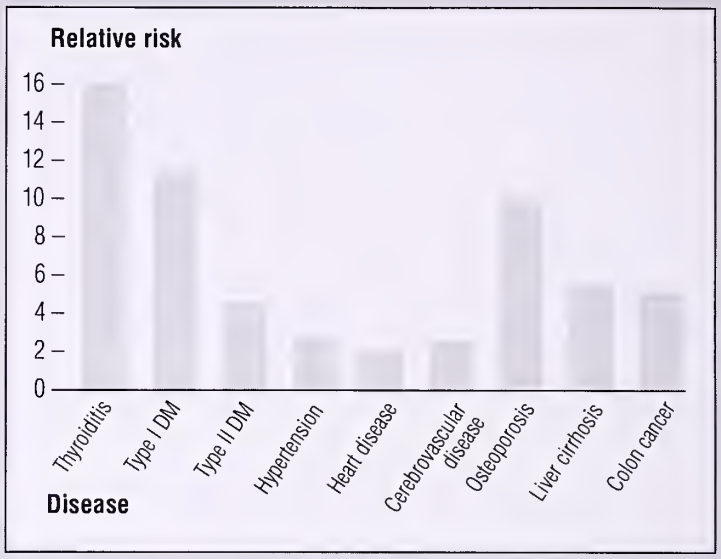
OVERALL MORTALITY AND MORBIDITY

Relatively little was known about the natural history of women with TS for many years because they were lost to follow-up. In 1986, Price et al[2] brought to the attention of interested persons the *mortality* statistics, *mortality* ratios, life expectancy data, and causes of death for 156 TS patients in Edinburgh who had survived infancy and had been followed an average of 17 years. The data had been collected over 25 years for the Abnormal Karyotype Register in Edinburgh, which was established in 1959. The *reduction in life expectancy* was 12.5 years at age 1, 11 years at age 20, and 10 years at age 40. Fifteen deaths had occurred among the 156 patients observed over the 25 years, a considerable reduction in life expectancy compared with normal newborns. The fraction of TS patients alive at age 60 years was 68%, in contrast to 88% of the general UK population. Eight deaths resulted from diseases of the circulatory system, which will be discussed further in the section about the cardiovascular consequences of TS. The other 7 deaths were due to a broad spectrum of diseases, also discussed later.

Several groups of investigators, primarily in Europe, where medical care is more often closely regulated, have recently reported on the *morbidity* in TS. Gravholt et al[3] undertook a 10-year study (1984 through 1993) of all 594 TS women known to be living in Denmark during this period. The focus was on the primary diagnosis (1^0 Dx) of all hospitalizations. The observed number of admissions for a specific 1^0 Dx versus that expected in the general population is the relative risk for a specific admission and is an indicator of the presence of associated diseases in TS. These investigators found that while most of the associated diseases manifested themselves in childhood or adolescence, many went undetected; *thus, emphasis needs to be placed on screening repeatedly for subtle manifestations of associated diseases in adulthood*. Figure 1[3] is based on some of these data, as published in a review by Elsheikh et al,[4] concerning the relative risks of some of the associated diseases. Gravholt et al also reported a slightly increased risk for colon cancer for all TS patients and a risk for gonadoblastomas in TS patients with a Y chromosome. Therefore, removal of the gonads is recommended for individuals having a Y chromosome or chromosomal fragment containing the testis determinant *SRY* gene.

Garden et al[5] previously (1996) published data from Liverpool regarding undiagnosed *morbidity* in adult women with TS. The morbidities uncovered were those possible to identify by lipid assessment, evaluation of thyroid function, testing of gonadal status, measuring routine biochemical profiles, and determining bone mineral density (BMD) in patients previously diagnosed with and treated for TS. The patients were referred for their continuing care to a clinic where the studies were routine for first visits. Serum cholesterol levels >5.2 mmol/L were detected in a surprising 50%, and 29% had low-density lipoprotein (LDL) values >4.0 mmol/L. Twenty-eight percent had at least 1 abnormality of thyroid function. Two had hypothyroidism and 6 had compensated hypothyroidism (normal thyroxine [T_4] with elevated thyrotropin). Lumbar vertebral areal BMD (aBMD) was <100% of the age-matched reference range in 84%. Femoral aBMD was similarly depressed. Ninety-five percent of these women allegedly were still receiving estrogen replacement. The authors emphatically and logically concluded that *TS patients leaving the care of pediatricians need appropriate evaluation and assignment to a physician or physicians experienced with the consequences of TS*.

Figure 1
Relative Risk of Disease in Turner Syndrome



Women with Turner syndrome have a greatly increased risk of developing autoimmune thyroid disease, ischemic heart disease, cerebrovascular disease, hypertension, as well as type I and II diabetes mellitus (DM). They are also at risk of osteoporosis fractures, cancer of the colon, and liver cirrhosis.

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The associated diseases in TS patients discussed in this section, as well as other associated or component diseases of TS, are reviewed in detail in the remaining sections and are presented approximately in the priority of their occurrence in the TS population.

ENDOCRINE-ASSOCIATED DISEASES AND/OR SIGNS AND SYMPTOMS PRODUCING CONSEQUENCES IN TS ADULTS

Short stature (SS) is a major physical characteristic of TS that is generally apparent in early childhood but that may not be evident with respect to the patient's stature falling below the 3rd percentile until 5 or 6 years of age. A normal adolescent growth spurt does not occur even with estrogen supplementation. There is strong suspicion that a chondrodystrophic etiology is the basic cause of the SS. A candidate gene[6] on the X chromosome, *SHOX*, has

been implicated in the SS and Madelung wrist deformity associated with TS.

The 50th percentile for adult TS women on the TS growth chart is 143 cm (56.3 inches).[7] The 95th percentile is 154 cm (60.5 inches), and the 3rd percentile is 132 cm (52.0 inches). The 50th percentile for normally growing girls is 164 cm (64.5 inches). A 21-cm difference is present between normal adult women on the 50th percentile of the normal growth curve and TS adult women who fall on the 50th percentile. The adult height of TS women is proportional to the parents' heights. The mean parental height is a good guide as to the ultimate extent of the SS.

Although the basic growth disturbance is most likely not a result of hormonal deficiencies, the possibility of associated growth hormone (GH), T_4, or triiodothyronine (T_3) deficiency should be considered, as should renal acidosis, diabetes mellitus, ulcerative colitis, and Crohn's disease. Thyroid disease is the most frequent contributor to the innate SS.

GH given in pharmacologic doses, although expensive, increases the expected adult height. GH-treated patients in the National Collaborative Growth Study[7] had an 8.4-cm mean increase over their baseline projected adult height; subjects receiving both GH and oxandrolone had a 10.3-cm increase. Currently, studies are in progress in which TS children are begun on GH treatment in early childhood instead of at a minimum of 9 years of age, as in previous studies. It is possible that SS will be less of a consideration in TS patients in the future as a result of early diagnosis and treatment.

Hypogonadism is the next most frequent characteristic of TS. It results from involution in utero of the ovarian follicles, although some follicles may not become atretic for ≥10 years. The result is hypergonadotropic hypogonadism. The uterus and vagina develop normally, but the uterus appears infantile in those adults who have complete lack of endogenous estrogen secretion until appropriate exogenous estrogen is administered. Normal uterine structure results. Gonadotropin levels, particularly follicle-stimulating hormone (FSH) levels, are elevated, even in the presence of spontaneous menses, which occur in 5% to 10% of 45,X individuals and in 20% to 30% of those with mosaicism. Those menstruating tend to develop menstrual irregularity and dysfunctional uterine bleeding associated with premature ovarian failure. Ovulation and fertility are rare. In a review in 1990, Kaneko et al[8] reported a total of 138 pregnancies in 62 women who were recorded in the literature. Eighty-two of the pregnancies produced liveborn infants, 23 of whom had congenital anomalies. Ten of these 23 had chromosome abnormalities. In a study by Sylven et al,[9] 4 (all mosaics) of 49 TS women conceived. All 8 children had normal karyotypes. Since an increased incidence of nondisjunction for both sex chromosomes and autosomes occurs in all types of TS, prenatal screening for chromosomal anomalies is important in any TS woman who is pregnant.

In vitro fertilization using donor oocytes has been successfully utilized multiple times in the last 10 years. Preparation of the uterus requires significant attention, but TS women can be optimistic when using assisted reproductive technologies at centers with excellent records for success. Women who are ovulating can probably enhance their chance of pregnancy by oocyte removal, in vitro fertilization, and implantation. Multiple oocytes and/or embryos sometimes can be organized, preserved, and reintroduced into the TS patient, but only 1 implant should be placed in the uterus per pregnancy because such pregnancies are considered high risk.

The goal of estrogen and progesterone therapy is to correct the sex steroid deficiencies in a manner that optimizes height potential, permits attainment of normal bone mass,

CME CERTIFICATION

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Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

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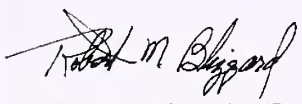
The Editorial Board invites all readers to write to the Editorial Board with suggestions for improving the publication. Write in care of Dr. R. M. Blizzard, 1224 West Main Street, Suite 701, Charlottesville, VA 22903. The Board also requests that you express your gratitude to those associated with Genentech, Inc. and Schwarz Pharma, Inc. for their sponsorship of what we hope you believe is an outstanding educational publication.



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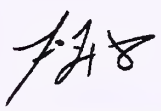

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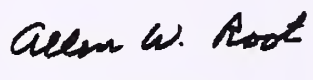


Allen W. Root, MD

and provides feminization with minimal risk of adverse effects. The available data suggest that treatment should be initiated between 12 and 14 years of age. Estrogen is generally started in a dose lower (0.325 mg conjugated estrogens or the equivalent) than necessary for adult replacement, and the dose is advanced every few months until the onset of menses. Cycling with progesterone (eg, oral medroxyprogesterone acetate 10 mg from days 16 through 25) is used to induce normal menstrual cycles and reduce the likelihood of uterine malignancy in the future. A full adult dosage (0.625 mg conjugated estrogens or the equivalent) should be established and daily estrogen given continuously. Determining how rapidly to increase the estrogen dose to achieve full replacement requires some individualizing, taking into consideration the height attainment desired, the patient's psychological need for feminization to occur, and the degree of osteoporosis present.

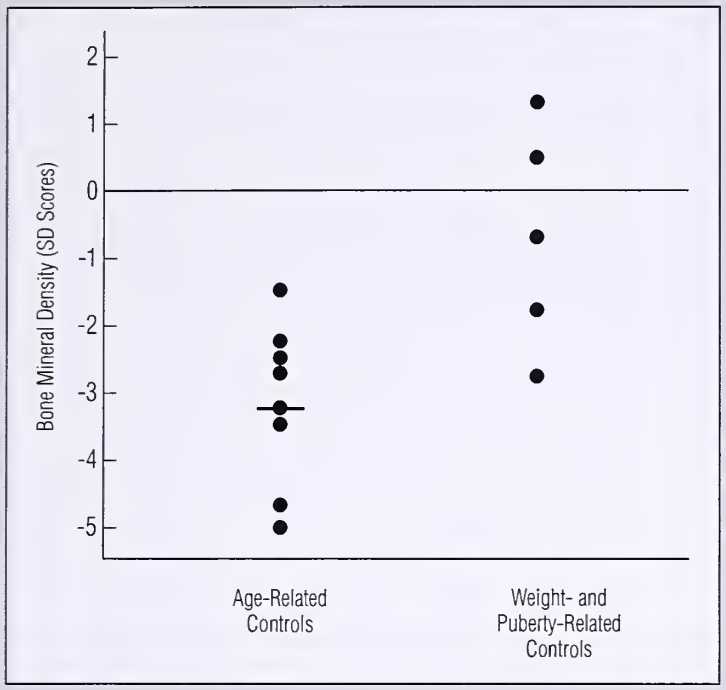
The duration of sex hormone replacement in adulthood required to minimize the adverse consequences resulting from sex hormone deficiencies is not clearly defined. It is now recognized that estrogen is desirable for prevention of osteoporosis and cardiovascular disease. Converging evidence based on the studies of older women suggests a potential benefit of estrogen on mood and cognitive function, including both verbal and nonverbal memory, of postmenopausal women.[10,11] Since estrogen replacement in adult TS women also normalizes body composition, physical fitness, and liver function,[12] one can make a strong case that all patients with TS should be on long-term steroid therapy (ie, at least until 50 or 60 years of age). Consideration of long-term estrogen therapy necessitates that the risk-to-benefit ratio continues to be analyzed. This is an ongoing process involving the choice of compounds, the route of administration, the dose, the schedule, and the outcome variables to be monitored under research protocols.

Questions concerning possible androgen replacement therapy have been raised since androgen production is decreased in TS as a result of atrophic ovaries. Currently, studies are under way exploring this issue and no specific recommendation can be made at this time.

Osteoporosis is reported to be very frequent in TS women. Controversy exists as to the etiology.[13] Among the possibilities are estrogen deficiency, a primary structural defect of bone, a derangement in the GH/insulin-like growth factor (IGF-I) axis, and/or a methodologic error in calculating BMD. aBMD is used in evaluating BMD in childhood instead of volumetric BMD (vBMD) because it is less dependent on the size and shape of the bones and the body.[14] Unequivocally, adult TS women have an increased incidence of various orthopedic problems, including scoliosis and fractures of the spine, metacarpals, and all long bones of the extremities. Ten percent to 15% of TS women require bracing or surgery. The relative risk (RR) for osteoporosis is 10, and that for fractures 2. Interestingly, collapse of vertebrae is an infrequent occurrence.

The effects of GH and estrogen have been evaluated. Lanes et al[13] compared aBMD of the lumbar spine and femoral neck in patients before beginning estrogen therapy at adolescence and after a mean of 6.1 years of significant estrogen treatment. The aBMD, as plotted for age,

Figure 2
Bone Mineral Density



Bone mineral density of the lumbar spine represented as standard deviation (SD) scores compared with age-, weight-, and puberty-related controls. The mean is shown by the short horizontal bar.

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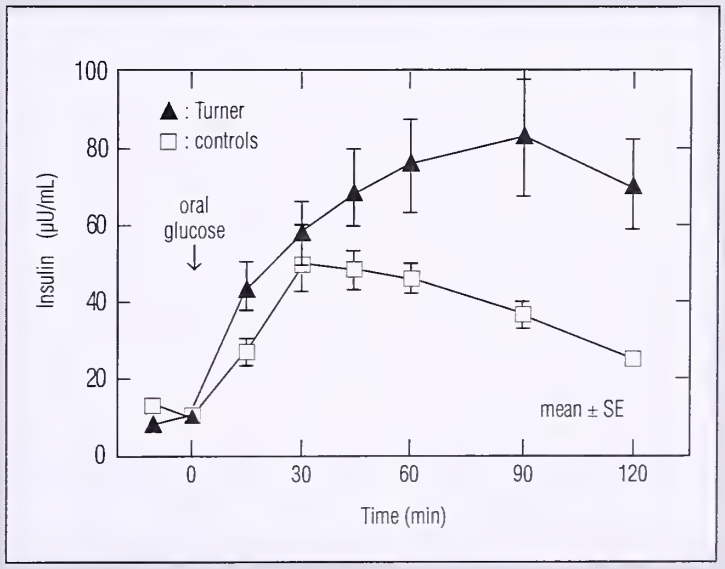
weight, and puberty-related controls, was reduced initially (Figure 2)[13] and did not change with treatment. Bertelloni et al[14] studied the vBMD in young TS women treated with estrogen replacement therapy (ERT) or ERT plus GH. In this well-designed study, the investigators reported that TS patients on ERT from adolescence had normal vBMD values in young adulthood and that their bone densities were related to their years on ERT. Higher vBMD values in patients started on ERT before the age of 14 years could not be confirmed. The authors also noted a difference between the ERT group and the ERT plus GH group that suggested that GH combined with ERT enhanced aBMD. However, this apparent increase in aBMD may be related to the increase in height. This would result because of the influence of height on the calculation and not because GH induced a significant true increase in the collection of bone mass. vBMD differences were minimally increased in this study, but Bertelloni et al stated that vBMD differences possibly could be significant if larger numbers of patients were studied. One of the authors' conclusions was that "since these data and those of others show a methodologically related low aBMD but a normal corrected vBMD, it is unlikely there is an intrinsic feature in TS of bone demineralization."[14]

The conflict of data and interpretations will be resolved only with collaborative efforts, that is, by sharing data and rethinking discrepant interpretations. However, from a practical viewpoint, ERT and GH therapy are both desirable as both have a long-known positive effect on bone structure, and estrogen prevents the development of postmenopausal osteoporosis. Consideration should be given to use of other antiosteoporotic drugs in adult TS women with previous osteoporosis and/or fractures.

Diabetes mellitus, hyperinsulinemia, and insulin resistance have an increased incidence in adult women with TS. Diabetes mellitus type I (insulin-dependent; IDDM) was reported in 9 of 594 TS women in Denmark[3] for an RR of 11.56 as compared with the general population of women. The RR for type II (non-insulin-dependent) diabetes mellitus (NIDDM) was 4.38. Previously, type I diabetes, which by definition requires insulin dependence, reportedly has not been increased, nor have islet cell antibodies, which reflect autoimmune diabetes mellitus, been found in increased incidence in IDDM. The Danish population may differ from most other populations in this respect.

NIDDM, insulin resistance, and hyperinsulinism in the TS population is well recognized (Figure 3).[15] Abnormal glucose tolerance, in association with a 2-fold risk of NIDDM, has been detected in up to 50% of TS women even before ERT.[3,15] A positive correlation between insulin resistance and an increased weight over percent ideal body weight is consistent with the well-known association between increased body mass index (BMI) and insulin resistance.[15] BMI standard deviation (SD) scores are increased starting in adolescence in TS women who have or have not received GH.

Figure 3
Insulin Resistance



Insulin release following stimulation by oral glucose in24 adult TS women (filled triangles) and 10 control women (open squares). In the Turner group, insulin release is significantly elevated (P <0.006) and the secretory response delayed peak concentration reached after 90 minutes versus 30 minutes in controls.

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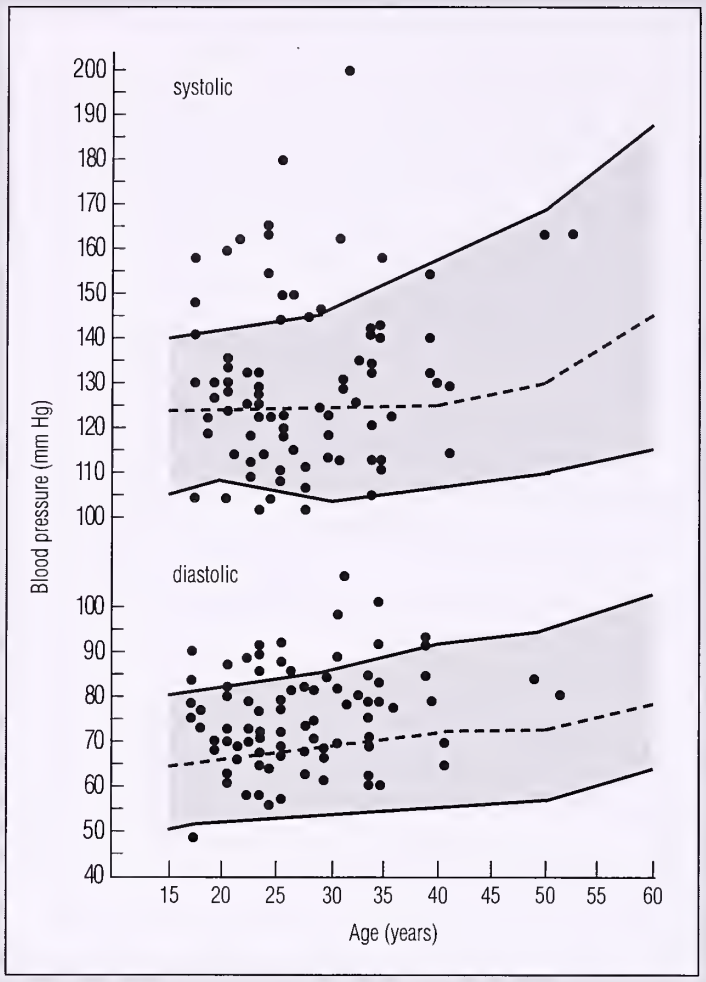
Figure 4
Distribution of Blood Pressure in TS



Shaded areas 5th to 95th percentile for UK population. Using diastolic BP of 90 mm Hg only 5 (5.5%) would be classified as hypertensive. With age-related percentiles 17 (15.5 %) were hypertensive. A similar underestimate of hypertension is observed with systolic blood pressure.

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TS women are at increased risk for developing *syndrome X (insulin resistance, hypertension, obesity, and hyperlipidemia)*. Hyperlipidemia characterized by hypercholesterolemia also starts at adolescence and often precedes estrogen administration. Up to 50% of TS women have been reported to have hyperlipidemia.[15] Hypertriglyceridemia also is frequent and may be a component of syndrome X.

The incidence of *hypertension* is increased (Figure 4).[16] More than 30% of TS women have a systolic and/or diastolic pressure above the 95th percentile.[16] Nathwani et al[17] reported that more than 50% did not have the normal diurnal variation in blood pressure. Hypertension was independent of obesity in one study.[16] The hypertension in TS is generally idiopathic rather than secondary to vascular anomalies, although this should be considered. The idiopathic hypertension is probably related through as yet undefined mechanisms to the BMI and insulin resistance, and it may be underecognized because of the failure to compare pressure readings with age-matched normal ranges (Figure 4).[16] Medical intervention with optimal control of elevated blood pressure is essential for prevention of the cardiovascular sequelae of hypertension.

Autoimmune thyroid disease is very common in TS but is infrequently associated with other autoimmune endocrine diseases.[4,15] Frequently, antithyroglobulin or antiperoxidase antibodies, or both, are elevated. More than 35% of adult TS women will have clinical or subclinical thyroid disease. Chronic lymphocytic thyroiditis, hypothyroidism, and thyrotoxicosis occur in that order of frequency. Screening with both types of antibody tests and palpation of the thyroid are strongly recommended every 2 years in adult TS women. The presence of goiter with or without thyroid antibodies or abnormalities of thyrotropin or T_4 is an indication to use levothyroxine therapy to prevent subtle hypothyroidism from occurring.

NONENDOCRINE-ASSOCIATED DISEASES AND/OR SIGNS AND SYMPTOMS PRODUCING CONSEQUENCES IN TS ADULTS

Cardiovascular diseases, both congenital and acquired, are important causes of mortality and morbidity in adults. Several review and original references are provided for readers who wish to expand their perspective.[2-5,16,17] Patients with TS have an increased risk of congenital malformations of the cardiovascular system, particularly of the heart. Coarctation of the aorta is the most common anomaly, occuring in 15% to 30% of patients, but often it is not clinically important. A positive correlation has been made between webbing of the neck and aortic coarctation. Patients with the 45,X karyotype are predominantly affected, as is true for other congenital anomalies. Approximately one third of TS patients have bicuspid aortic valves and one quarter have mitral valve prolapse. Cardiac physiologists believe that the bicuspid valve permits a jet of greater than normal pressure against the ascending aorta, and atherosclerosis results. Affected patients should receive prophylactic antibiotics at times of dental or other surgery. All TS patients who were not previously evaluated with cardiac ultrasound should be tested periodically.

Aortic aneurysms occur with and without aortic coarctation. Cystic medial necrosis has been reported in patients with dissection, which suggests that a more generalized congenital vascular dysplasia may exist. This could account, at least in part, for the increased incidence of strokes and cerebrovascular disease. Other contributing factors to vascular disease in various patients are hypertension, obesity, and insulin resistance. The increased risk of heart disease and atherosclerosis in TS is consistent with the recent finding from death certificates "demonstrating that approximately 50% of all deaths were caused by cardiovascular disease occurring 6 to13 years earlier than expected."[1]

Aortic root dilation occurs with a reported prevalence of between 8% and 28%. Dilation or dissection may occur at any age. *The risk of dissection in the presence of dilation may be as high as 60%. Therefore, regular surveillance of adult patients with TS is recommended.* Magnetic resonance imaging (MRI) should be used if aortic root dilation is detected by ultrasound to assess the severity and provide more precise measurements for follow-up.[4] Coronary heart disease may be twice as likely to occur in TS patients compared with matched controls, according to an extended study reported by Gravholt et al.[3] However, the true incidence of ischemic heart disease in adult TS women remains unknown. These women have several risk factors that make them candidates for premature coronary thrombosis and/or ischemic heart disease. These risk factors include insulin resistance, hyperlipidemia, hypertension, estrogen deficiency, and obesity. For practical purposes, these women should be considered at high risk for coronary thrombosis as well as other vascular diseases.

Less life-threatening vascular malformations that have been reported include hemangiomas, intestinal telangiectasia, venous ectasia, lymphangiectasia, and gastrointestinal lymphangiomas, which can produce protein-losing enteropathy. Lymphedema of the hands and feet, another vascular anomaly, sometimes persists into adulthood and is a site of skin breakdown with superimposed infection, a recurrent aggravation to those who have it. Treatment is difficult.

Partial or extensive *deafness* plagues adult TS women. Sixty-one percent had some hearing loss in one study and 27% required hearing aids, which is probably an underestimate.[9] The hearing loss may be conductive and/or sensorineural. Because of the anomalous eustachian tubes in TS children, multiple ear infections contribute to the deafness in adulthood. The sensorineural loss appears to have other genetic determinants. A characteristic sensorineural dip in the midfrequency range is frequently identified. Unfortunately, this type of sensorineural hearing deficit results in hearing speech poorly, does not lend itself to improvement with hearing aids, and has been related at times to diminished psychological well-being in TS. Audiologic evaluation and follow-up by an otolaryngologist, as indicated by the initial exam, is highly desirable.

Renal anomalies (and resultant disease) are particularly frequent in those with the 45,X karyotype. Between 30% to 60% of TS patients have anomalies such as horseshoe kidneys, double collecting systems, and malrotation. Six percent to 10% of adult TS women may have silent hydronephrosis. Consequently, *ultrasound studies are indicated at least once in adult life and should be repeated 10 years later* even if no silent hydronephrosis was observed the first time. If the collecting system is found to be abnormal, regular screening for urinary tract infection should be initiated. The hypertension observed frequently in TS is seldom of renal etiology, but this possible cause should always be in the differential diagnosis.

Skin anomalies in TS adults include an increased tendency to extensive keloid formation, which must be taken into account when considering cosmetic surgery on the face and/or a webbed neck. Extensive pigmented nevi occur in late adolescence or early adult life, but malignant degeneration does not seem to be a problem. The residual lymphedema mentioned previously can be a difficult problem to treat.

Hepatic and gastrointestinal disease need to be considered in adults with TS, although life-threatening complications are believed to be only minimally increased.[9] Cirrhosis, hepatitis, and colon carcinoma are not mentioned in the review by Hall and Gilchrist[1] or the article by Price et al[2] dealing with mortality ratios, life expectancy, and causes of death in patients with TS, although nonspecific inflammatory disease of the bowel had been reported by Price in 1979.[18] Gravholt et al[3] reported an RR of 5.69 for cirrhosis and 2.25 for ulcerative colitis. Elevated liver enzymes have been reported in children and adolescents even before ERT, and in one report there was a conspicuous rise in serum liver enzyme levels occurring with treatment with conjugated estrogens.[19] No essential morphologic equivalent was found in liver sonography or biopsy specimens in a population of tall females receiving a 6-fold larger dose. Wemme et al[19] concluded that since the underlying mechanism is unknown, the possibility of an adverse long-term effect cannot be ruled out. They conjectured that using transdermal estrogen therapy may be preferable to oral or injectable estrogen, as the latter passes in large doses through the portal vein into the liver, while transdermal estrogens do not.

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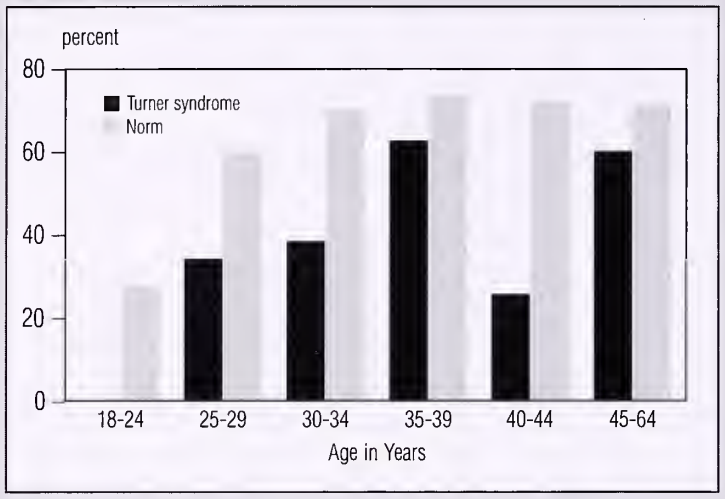
Figure 5
Percent of Turner syndrome women married, by age group, as compared with US census (1992) data.



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Neurodevelopmental characteristics of TS include impaired visuospatial abilities, leading to poor scores on nonverbal IQ exams and academic difficulty with mathematics and geography. These observations led to reports of frequent mental retardation. However, as Hall and Gilchrist[1] stated in their review, the presence of moderate to severe mental retardation is either not increased or increased only slightly in TS. On full-scale IQ testing, the total IQ is average or above. In 1995, Ross et al[20] reported concerning the neurodevelopmental changes from childhood through adolescence in 56 TS girls. The results demonstrated consistent findings across the age ranges studied (6 through 14 years). Verbal and language skills resembled those of 100 control girls. Performance IQ was relatively depressed, as were tests of visuomotor skills and attention. TS subjects also showed evidence of multifocal or diffuse right cerebral dysfunction. The constellation of neurocognitive deficits observed in TS is most likely multifactorial and relates to a complex interaction between genetic abnormalities, hormonal deficiencies, and other unspecified determinants of cognitive ability.

Several groups followed TS women through adulthood and reported increased social isolation and low self-esteem.[9,21,22] In adulthood, TS women were less likely to date or be married or to be involved in a sexual relationship. Sylven et al,[9] reporting on 49 adult TS women, determined that 31 had been or were married, 6 had become divorced, all had completed elementary school, 8 had university degrees, and 46 were employed. In an excellent study, Pavlidis et al[22] confirmed these data in a series of 80 adult TS women. Thirty-six (45%) reported seeking professional help primarily because of depression or, to a lesser extent, stress/anxiety, independence issues, help in coping with TS, or shyness. Sixty-eight (85%) were employed with 52% in professional positions. Forty-three (54%) were married (Figure 5). Of the entire group, 45% had not experienced intercourse. *In working with these women, their strengths should be emphasized and their limitations such as visuospatial deficits recognized so that unreasonable expectations are not made by their teachers, employers, parents, and spouses.*

CONCLUSION

Women with TS should not be regarded as a group but as individuals with different skills and abilities, and they should be treated according to age and not height. They want to know about TS; and if one does not tell them, they will go to the library or the Internet to find out for themselves, and often obtain incorrect information. Clearly, patients and physicians need to enhance their knowledge about the variety of medical and social problems associated with TS.[9] In conjunction with this approach, this author wishes to encourage the utilization of contact groups such as the the Turner's Syndrome Society of the United States (http://www.turner-syndrome-us.org/).

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In Future Issues

Circadian Rhythms and the Genes and Clinical Conditions Related to These: Scott A. Rivkees

Androgens in Puberty: Role in Metabolism and Growth: Nelly Mauras

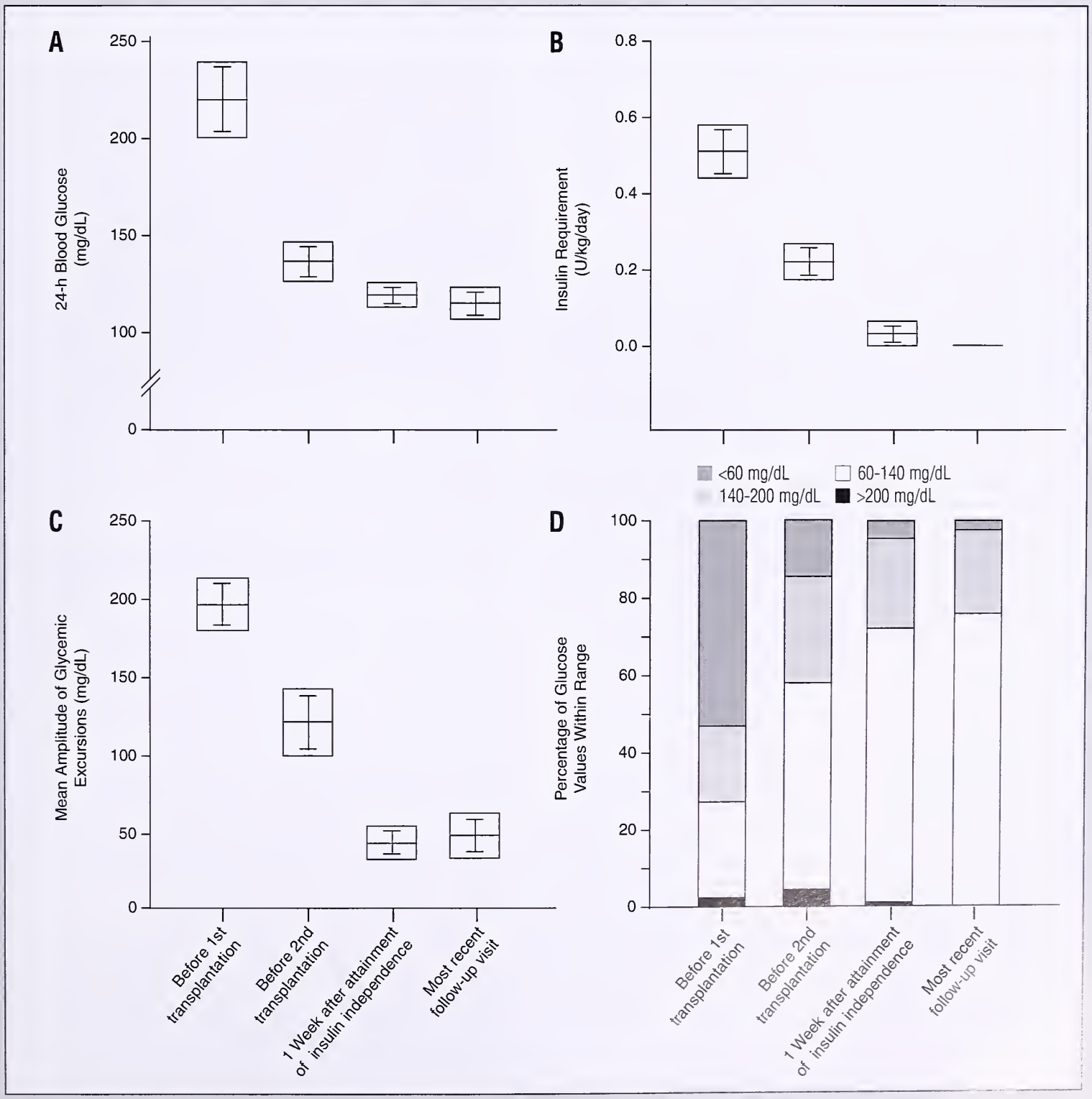
Endocrine Complications of the Successful Treatment of Neoplastic Diseases in Childhood: Charles Sklar

Islet Transplantation in Seven Patients With Type I Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen

This very important study and report records a significant advancement in islet cell transplantation of tremendous potential. The apparent success of exceeding the usual limited success of islet transplantation is attributed by the authors, first, to transplanting an adequate number of islet cells and, second, to the replacement of glucocorticoids as the immunosuppressive agents with more recently designed nonsteroidal immunosuppressive agents.

The subjects were 7 consecutive patients with type I diabetes of more than 5 years who had essentially no stimulated C-peptide, whose glucose concentrations remained uncontrolled despite insulin therapy, and who had recurrent severe hypoglycemia. The new immunosuppressive agents that were used were *sirolimus* at the usual doses, low-dose *tacrolimus*, and *daclizumab*, which is a monoclonal antibody against the interleukin-2 receptor. The islet cell infusions required 2 sep-

Figure



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arate transplants in 6 patients, and 3 in 1 patient. The percutaneous transhepatic approach was used to gain access to the portal vein into which the islet cells were infused and transported into the liver. The quantity of insulin-producing cells transplanted is approximately double that reported previously.

Not only did the patients have essentially no insulin requirements for the time intervals of follow-up (4.5 to 15.0 months), but they also had no hypoglycemic episodes. The resultant 24-hour blood glucose, insulin requirements, mean amplitudes of glycemic excursions, and percentage of glucose values within normal range are demonstrated in the figure. Toxicity over the short term (up to 15 months) was limited to the requirement for blood transfusions following islet cell infusions (corrected by experience by developing a gel foam pad to be placed with the infusion) and minor superficial ulcerations of the buccal mucosa that resolved after the dose of *sirolimus* was reduced and the capsule formulation of *sirolimus* was substituted for the liquid form. No cytopenia resulting from *sirolimus* was observed. There was effective immunosuppression with no apparent diabetogenic or significant toxic effects, and no evidence of graft rejection, which has been a problem in transplants previously performed utilizing earlier methods and agents.

Shapiro AMJ, et al. *N Engl J Med* 2000;343(4):230-238.

Editor's comment: *This pilot study undoubtedly will lead to other studies that stand a good chance of confirming these rewarding preliminary results. Patient selection was such that the patients were desperately in need of help to control their diabetic symptomatology but had no significant secondary complications such as significant renal disease. Hopefully, this procedure will lead to an acceptable and readily available method of treatment for type I diabetic patients regardless of various parameters associated with the basic disease. The utilization of an acceptable nonorgan transplant for adolescents and possibly preadolescents stands a good chance of stabilizing the erratic glucose levels that lead to so many problems in adolescent patients, whose self-images deter them from taking insulin on a regular basis. Endocrinologists are inundated when diabetic patients, for many different reasons, fail to adhere to their treatment regimen. The authors point out that availability of cadaver pancreases is greater than one might think. Fewer than one third of such available pancreases are actually transplanted. Therefore, islet cells can be made available to a significant extent.*

The article's concluding paragraph is worth noting: "In patients with type I diabetes, glycemic control can be achieved with intensive insulin therapy and pancreatic transplantation. Intensive insulin therapy does not normalize glycosylated hemoglobin values and may cause severe hypoglycemia. Pancreatic transplantation provides excellent glycemic control, and although the outcome of the procedure has improved dramatically, it remains an invasive procedure with a substantial risk of morbidity. The findings published here indicate that islet transplantation alone is associated with minimal risk and results in good metabolic control with normalization of glycosylated hemoglobin values, and with sustained freedom from the need for exogenous insulin."

Robert M. Blizzard, MD

Hypoglycemia: A Complication of Diabetes Therapy in Children

Because of their erratic activity and eating behavior, hypoglycemia in diabetic children is much more difficult to predict and, therefore, to prevent than pediatricians wish to tolerate. The consequences of hypoglycemia are the greatest in this youngest age group, where these problems are paramount. The authors focus on the whys, the wherefores, and the treatment, since hypoglycemia is the most common acute complication in insulin-treated type I diabetic patients. The younger the patient, the greater the frequency of both mild and severe hypoglycemia. Tighter glycemic control also is associated with increased frequency of hypoglycemia. Conversely, however, people with poor metabolic control whose glycosylated hemoglobin levels are high also are susceptible to severe hypoglycemia. Does hypoglycemia matter? The authors answer with a resounding yes! Symptoms are uncomfortable and carry the fear of loss of control or unconsciousness. Morbidity occurs frequently, and mortality sometimes occurs. In addition, sometimes the patient's fear of hypoglycemia is greater than the fear of future microvascular complications.

Previous and repeated mild hypoglycemia can induce hypoglycemia unawareness, thereby leading to diminished warning symptoms and impaired hormonal counterregulation. The authors state that even mild hypoglycemia should be considered as having potentially dangerous consequences.

Following this introduction, they discuss the prevalence of hypoglycemia and begin by establishing definitions they believe should be used for "severe hypoglycemia." Some have defined the entity as an event that causes coma or seizures, while others have defined it as any episode that requires external assistance. The authors recommend that severe clinical hypoglycemia should include only episodes of unconsciousness because these can be ascertained consistently across all age groups, which is not possible with a less intense definition. "Mild chemical hypoglycemia" has been defined by some as glucose values below 54 mg/dL but not

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GROWTH, Genetics, & Hormones
Volume 17, Number 1

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1. a b	3. a b	6A. a b c
2A. a b c	4. a b c d e	6B. a b c
2B. a b c	5. a b c	7. a b c d

Course Evaluation

5 = Excellent 4 = Above average 3 = Good 2 = Below average 1 = Poor

Please evaluate this course with respect to the following:

1. Educational value of newsletter	5	4	3	2	1
2. Clinical relevance of articles	5	4	3	2	1
3. Newsletter style/format	5	4	3	2	1
4. Length of articles	5	4	3	2	1
5. Were the educational objectives met?	5	4	3	2	1

6. In your opinion, how could this newsletter be improved?

_____

_____

_____

below 40 mg/dL, whereas others use the cutoff glucose level of 65 mg/dL. The authors argue that 60 to 65 mg/dL (3.3 to 3.6 mmol/L) should be used to define "hypoglycemia" whether the patient is symptomatic or not. Unquestionably, severe hypoglycemia is more frequent in adolescents than in adults, as was demonstrated in the diabetes control and complications trial. This was true whether the patients were in the intensive or conventional treatment groups. Data regarding the number of episodes of coma/seizure and also on moderate hypoglycemia per 100 patient-years were considered. The data are well worth reviewing in the original article, particularly by those who deal with diabetes frequently in their practice. The greatest frequency of severe hypoglycemia was found in children <6 years of age. By the fourth year of the study, this group had 42 events per 100 patient-years. This means that of 100 patients having the disease over a 1-year period, there would be 42 severe hypoglycemic episodes.

The authors consider under the causes errors in treating hypoglycemia, the pharmacokinetic and physiologic differences in children with diabetes, and hypoglycemic unawareness. Considering the consequences, they discuss symptoms, changes in mental efficiency, and chronic brain dysfunction. In considering prevention, they state the key to prevention of severe hypoglycemia and associated complications is prevention of even mild episodes, which requires regular glucose monitoring, and the development of protective strategies on the part of the diabetic patient and family. Insulin regimens and diet and exercise also are considered in this section.

Becker DJ, Ryan CM. *Trends Endocrinol Metab* 2000;11:198-202.

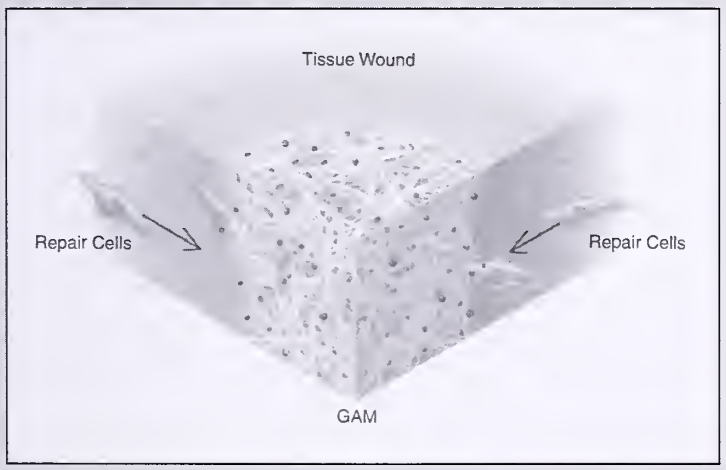
Editor's comment: *This article emphasizes the problems of insulin therapy in childhood. It follows the previous abstract (Shapiro et al) because of the potential relationship in future treatment of using islet cell transplants. If the reader has not read this article by Becker and Ryan and is treating children with diabetes, I strongly recommend that he/she do so.*

Robert M. Blizzard, MD

Who Wants to Be a Tissue Engineer?

Tissue engineering is a hot topic and not foreign to *GROWTH, Genetics, & Hormones* since many genetic disorders could potentially benefit from regenerated tissues and since tissue regeneration involves local growth and its hormonal control. Successes have been limited in stimulating regeneration of mammalian bone, skin, blood vessel, and spinal cord when biomaterial scaffolds, which hopefully might bridge tissues to be regenerated and promote cell migration, proliferation, and differentiation, are used. While attractive, the use of growth factors to enhance regeneration has been hampered by difficulties in selectively delivering potential therapeutic agents at proper concentrations and for extended periods.

Figure 1



The schematic figure shows a GAM implant in a fresh wound site (*inner area*). A GAM at its most basic consists of 2 ingredients: plasmid DNA and a structural matrix carrier. As part of the wound healing response, granulation tissue fibroblasts proliferate and migrate from viable tissue (*outer area*) surrounding the wound into the GAM. Once there, fibroblasts take up and transiently express plasmid DNA. The GAM matrix has 2 functions: It holds plasmid DNA in the wound site (until cells arrive), and it acts as scaffolding that promotes fibroblast ingrowth and accumulation near the DNA. While in the matrix, transfected fibroblasts act as local in vivo bioreactors, producing plasmid-encoded proteins that stimulate wound repair.

Reprinted with permission from Bonadio J. *J Mol Med* 2000;78:303-311.

Bonadio and coworkers now offer a novel approach to local tissue engineering. The basic concept offered is to introduce plasmid DNA encoding the therapeutic factor into a biodegradable porous scaffold that is implanted into the region where regeneration is desired. The delivery system is called "gene activated matrix," or GAM (Figure 1). As cells grow into the scaffold, they take up the plasmid, express the plasmid DNA, and synthesize the recombinant therapeutic factor. Eventually, the scaffold is degraded as new tissue is formed.

At first glance, this seems too good to be true. However, Bonadio provides evidence that it works. Using wound healing as a model, the group has shown that fibroblasts growing into granulation tissue take up and express recombinant protein for weeks. Referring to earlier work using a canine bone defect model (Figure 2 on page 12), he notes that bone healing is much improved over controls by implantation of GAM-containing plasmids encoding BMP 4 or PTH fragment 1-34, and that the therapeutic effect is enhanced when the 2 plasmids are used together. The results suggest that GAM provides a dose-dependent, reproducible, and safe strategy for stimulating tissue regeneration.

After discussing the rationale for using GAM in wound healing and reviewing experiments with animals, Bonadio turns his attention to how GAM might be used in human medicine. He suggests that the first use of the approach may best be in situ-

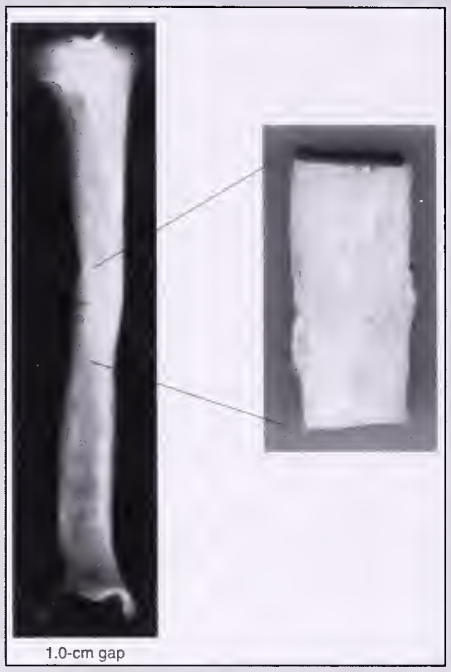
ations in which wound healing is inadequate. He describes the rationale and strategy for using GAM containing PTH 1-34 plasmids to treat hip fracture in elderly individuals with osteoporosis—an exciting postulate.

Bonadio J. Tissue engineering via local gene delivery: update and future prospects for enhancing the technology. *J Mol Med* 2000;78:303-311.

Editor's comment: *It is a long way from elderly osteoporotic patients with hip fractures to children with growth disturbances, but the principles involved in locally delivering plasmids encoding potentially therapeutic genes, as outlined in this article, may be applicable to a variety of disorders of interest to the* GGH *readership, especially for treatment of localized growth disturbances. The GAM technology is still in its infancy and remains to be proven safe and effective in humans, but the results presented to date are very encouraging. It is important to stress that determining which growth factors or, more likely, which combinations of growth factors are most effective for different clinical situations remains as big a challenge as developing the means to deliver such factors. The concept of being a tissue engineer may have much potential. After you read Bonadio's review you may agree.*

William A. Horton, MD

Figure 2



The figure shows evidence of complete healing of canine bone defect. *(Far left)* Radiograph shows a tibia with repaired segmental defect following GAM implantation (1.0-cm gap). *(Left)* Morphologic view shows 1.0-cm defect filled in with new bone following plasmid gene transfer.

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Long-Term Effect of Bone-Marrow Transplantation for Childhood-Onset Cerebral X-Linked Adrenoleukodystrophy (X-ALD)

The authors report that bone marrow transplantation (BMT) undertaken at the inception of neurologic symptoms in children with X-linked adrenoleukodystrophy (X-ALD) often can halt or reverse the progressive neurologic disease characteristics of this illness. However, the component of primary adrenal failure progresses. Eighteen boys aged 5.3 to 11.8 years with the slowly progressive form of cerebral disease or the advanced form of cerebral disease of X-ALD underwent BMT.

Six transplanted subjects died: 2 of complications of BMT, 2 with advanced cerebral disease, and 2 with slowly progressive cerebral disease that accelerated to advanced cerebral disease after BMT.

Twelve patients survived. Eight patients are in regular school classes; 1 has graduated from high school and attends college. The plasma concentrations of very long chain fatty acids (VLCFAs) decreased in all subjects after BMT. Magnetic resonance imaging (MRI) revealed decreasing myelinization for 1 to 2 years after transplantation; it then stabilized and even increased in 3 patients. Clinically, in 5 patients with mild corticospinal signs, resolution occurred in 3 and remained stable in the other 2. In 2 subjects, seizure control was greatly improved. Vision deteriorated in 3 patients. Verbal IQ (VIQ) scores remained stable after BMT in 10 of 12 subjects. In 5 of 11 patients tested, performance IQ (PIQ) increased by >10 points. In 4 of the 11, PIQ decreased significantly but then stabilized. Language skills, auditory processing, and motor performance increased appropriately over time in most patients. In the majority of a similar population of 13 boys with X-ALD for whom no compatible marrow donor could be found, 7 have died, 4 are in a vegetative state, and 2 became stable after an initial period of deterioration. The investigators conclude that BMT early in the course of neurologic disease can alter the natural history of X-ALD.

Shapiro E, et al. *Lancet* 2000;356:713-718.

Editor's comment: *The mutated gene (ALD, OMIM 300100) in boys with X-ALD encodes a peroxisomal membrane ATP-binding transporter protein that, when inactivated, impairs β-oxidation of fatty acids, resulting in accumulation of VLCFAs with 24 to 30 carbons. Esterified to cholesterol in the CNS and adrenal cortex, these compounds prove injurious to these tissues. Present data suggest that bone marrow cells cross the blood-brain barrier and attenuate the process(es) that lead to demyeliniation and neurologic deterioration in children with X-ALD.*

The authors made an additional educational contribution by classifying the severity of X-ALD patients into 4 clinical categories. This classification currently exists in general for X-ADL and goes beyond the characterizations in the 12 patients reported. There is clinical value in this classification, which is repeated here.

1. *Patients with no cerebral disease, with or without Addison's disease, in whom MRI and neuropsychological tests are normal. These are not candidates for BMT. About half of this group will develop neurologic signs involving the spinal cord in adulthood.*

2. *Patients with slowly progressive cerebral disease, with or without Addison's disease. MRI shows slow progression of demyeliniation. BMT is to be considered. Disease severity is evaluated by scoring the extent of demyeliniation on the MRIs and performance on neuropsychological tests. MRIs are scored using a demerit scale ranging from 0 to 34 devised by Loes et al. BMT is recommended for patients whose cognitive abilities exceed a VIQ or PIQ of 80.*

3. *Patients with stable cerebral disease. Included are patients with MRI and neuropsychological abnormalities at diagnosis and in whom follow-up shows no evidence of MRI and neuropsychological deterioration. Close monitoring is required to detect change that may signal decline. (Not stated, but implied, is that those who are declining but whose IQ remains >80 might be candidates for BMT.)*

4. *Patients with advanced cerebral disease. These include patients with rapid progression of disease who decline rapidly to a vegetative state and have marked VIQ or PIQ dysfunction (<80) and neurologic signs. Current methods of BMT are not beneficial.*

The authors also state: "The absence of any correlation between the clinical phenotype and the ALD *gene mutation or the biochemical defect, and the effectiveness of BMT ONLY at an early stage of the disease, lead us to recommend careful planning and frequent observation of all boys biochemically identified with X-ALD with normal brain MRI. No biological marker predicting the onset of cerebral demyelination is as yet available. Therefore continued MRI and neuropsychological testing are the only tools allowing the identification of patients who will benefit from BMT. Similarly no existing marker predicts whether or when a patient with a "slowly progressive cerebral disease" will enter into the "advanced cerebral disease" stage. Observations raised the hope that VLCFA could be decreased or even normalized by new pharmacological approaches. BMT, however, remains the only effective therapeutic approach in the cerebral form of X-ALD. The opportunity to recommend BMT at an early stage of cerebral X-ALD should not be missed."*

Allen W. Root, MD

Loes DJ, et al. *Am J Neuroradiol* 1994;15:1767-1771.

Transmission of BSE (Bovine Spongiform Encephalopathy) by Blood Transfusion in Sheep

Houston et al published an early warning report before completion of a study that they were doing to look at cross-species transmission of bovine spongiform encephalopathy (BSE) through blood transfusion. This study was aimed at answering the question of whether there is a concern about blood transfusions transmitting the variant Creutzfeldt-Jakob (vCJD) disease in Britain from anyone living in Britain or who traveled in Britain between 1980 and 1996. Several countries have banned blood donations from people who spent time in Britain during the time of potential exposure to BSE.

Houston et al were engaged in a study to see if it is possible to transmit BSE between sheep by blood transfusion after the blood donor sheep had orally ingested the infecting agent. It turns out that sheep blood types are very complex, so this study was not a simple matter. It had been thought that there was a barrier to cross-species transmission of infectious agents. BSE-infected sheep harbor infection in peripheral tissues (tonsils, for example) prior to becoming symptomatic and thus are similar to humans infected with vCJD. A group of sheep were orally challenged with 5 g of BSE-affected cattle brain. At a later time, their blood was taken and transmitted into scrapie-free sheep. For the most part, whole blood was used for the transfusions and only a single transfusion was made. BSE clinical signs and pathologic changes have occurred in 1 of the sheep who received blood from a BSE-infected animal who was asymptomatic at the time of the transfusion. The donor had been challenged by oral BSE cattle brain 318 days before whole blood was taken. The BSE developed in the recipient animal 629 days after the transfusion. This suggests that the blood was taken from the orally challenged sheep halfway through the incubation period and yet it was nevertheless able to infect the recipient sheep.

This experiment does indicate that BSE can be transmitted between individuals of the same species by whole blood transfusion and thus has implications for the blood transfusion system in general. The United Kingdom has been utilizing leukocyte-depleted blood; however, this may not be sufficient to avoid the problem.

A number of models have been utilized to predict the incidence of vCJD in the United Kingdom. There has been concern that as many as 500,000 individuals could become affected. The models have varying lengths of incubation and various calculations as to the number of people who would become infected and symptomatic after eating meat from an infected cow. The observed number of cases affected in early 2000 was 75 (Table). There appears to be a susceptible prion genotype, which is present in about 40%

Table
Annual Number of Onsets, Classifications, and Deaths From vCJD in the UK

Year	Onsets	Classified as vCJD	Deaths
1994	8	0	0
1995	10	7	3
1996	11	8	10
1997	14	12	10
1998	16	17	18
1999	16	17	14
2000	0	14	14
Total	75	75	69

Based on current classification criteria, applied retrospectively where appropriate.

Reprinted with permission from Andrews NJ, et al. *Lancet* 2000;481-482.

of the population. It is speculated that there are many consumers still at risk, but total vCJD mortality appears to be lower at this time than previously predicted.

Many pathologists have begun to screen tonsil and appendix tissue since they were found to be positive in 1 affected individual 8 months prior to the onset of vCJD symptoms. For practical purposes, no positive specimens have been found when doing population screening (~3,500 cases).

Andrews NJ, et al. Incidence of variant Creutzfeldt-Jakob disease in the UK. *Lancet* 2000;356:481-482.

Brown P. BSE and transmission through blood. *Lancet* 2000;356:955-956.

Dieter RS. Prion protein in tonsil and appendix tissue. *Lancet* 2000;356:505.

Ghani AC, et al. Predicted vCJD mortality in Great Britain. *Nature* 2000;406:583-584.

Houston F, et al. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000;356:999-1000.

Markham D. Prion protein in tonsil and appendix tissue. *Lancet* 2000;356:505-506.

Editor's comment: *HIV and hepatitis have led to concerns about the safety of the blood transfusion system. This new report about blood transfusion transmission of prion disease in sheep is quite worrisome. There has not been a single documented case of human CJD, such as observed following contaminated GH injection, that could be related to blood transfusion. Nevertheless, it is of great concern from the standpoint of screening and excluding potential donors of blood products. It took Houston et al 3 years to produce 1 vCJD-positive sheep. Although methodologies to minimize the risk of blood transfusion are improving, there still is concern about whether an epidemic could occur. The good news is that the number of people affected with vCJD seems to be less than was predicted. The good news also is that many lessons are being learned about transmittable diseases, which is important for future public health practices.*

Judith G. Hall, OC, MD

Effect of Growth Hormone Treatment on the Adult Height of Children With Chronic Renal Failure

Previous studies have demonstrated that GH therapy increases the growth rate and improves standardized height in prepubertal children with chronic renal failure. What has not been known, however, is whether such therapy actually improves final height. It has been speculated that GH therapy could accelerate the onset or progression of puberty and negate any effect of early prepubertal treatment. Haffner et al report for the German Study Group for Growth Hormone Treatment in Chronic Renal Failure their analyses of 38 initially prepubertal children with chronic renal failure who were treated with GH for 5.3 years until they reached their adult height. Their growth was compared with 50 matched children with chronic renal failure who were not treated with GH. Of note, the 50 children who did not receive GH had growth retardation that was less marked than that of the treated children.

All subjects in the study had chronic renal failure with a height SD of −2 or below and a height velocity below the 25th percentile during the year prior to the onset of treatment. The 38 children (32 boys and 6 girls) who were treated with GH were 10.4 ± 2.2 years at the initiation of GH and their bone age was 7.1 ± 2.3 years with an SD score of −3.1 ± 1.2. During the study, 11 of the children were started on dialysis and 9 subsequently received a renal transplant. GH was administered in a total weekly dose of 0.33 mg/kg body weight. Fifty children (31 boys) in the control group were matched with respect to age at first observation, underlying renal disease, treatment, residual renal function, and cumulative dose of glucocorticoids. They were not treated with GH because they had relatively little or no growth retardation at baseline. Standard anthropometric measurements were obtained at 3- to 6-month intervals during the study and bone age was determined by the Tanner-Whitehouse II (TW2) method approximately every 12 months. The genetic target was calculated as a midparental height +10 cm for boys and −2.6 cm for girls.

During the prepubertal observation period, height velocity in the GH-treated children increased over baseline and exceeded values in both the controls and in normal children. After the prepubertal peak, the height velocity decreased until the start of the pubertal growth spurt. The total height gained during the prepubertal observation period was twice as much as that

Table
Predictors of Growth During the Observation Period in the Growth Hormone-Treated and Control Children Combined

Period and Predictor	Effect	Partial R^2	Cumulative R^2	*P* Value
Prepubertal period (change in cm of height)				
Increased duration of prepubertal period	Positive	0.67	0.87	<0.001
Increased duration of growth hormone therapy	Positive	0.13		<0.001
Greater initial target-height deficit	Positive	0.04		<0.001
Greater % of time spent on dialysis	Negative	0.03		0.006
Pubertal growth period (change in cm of height)				
Increased duration of pubertal period	Positive	0.45	0.61	<0.001
Increased duration of growth hormone therapy	Positive	0.11		<0.001
Male sex	Positive	0.05		0.005
Total observation period (change in cm of height)				
Greater initial target-height deficit	Positive	0.68	0.78	<0.001
Increased duration of growth hormone therapy	Positive	0.06		0.002
Greater % of time spent on dialysis	Negative	0.04		0.004
Total observation period (change in standard deviation score)				
Increased duration of growth hormone therapy	Positive	0.58	0.64	<0.001
Greater initial target-height deficit	Positive	0.06		0.008

Reprinted with permission from Haffner, et al. *N Engl J Med* 2000;343:923-930.

in the control children. During puberty, peak height velocity was not significantly higher in the GH-treated children than in the controls. The onset of the pubertal growth spurt was delayed in these children by approximately 2½ years (compared with normal children) and the duration of the growth spurt was 1.6 years shorter compared with that of normal children.

The total pubertal height gain was similar in the GH-treated and the control children, but was 65% of that in normal children because the pubertal growth spurt was shorter.

Catch-up growth was sustained in the GH-treated children whereas the control children had progressive growth failure. The standardized height increased from the baseline mean of -1.4 SD. The mean final height was 1.6 SD below normal in the treated group, whereas in the control children the standardized height decreased by a mean of 0.6 SD to a final mean adult height of 2.1 SD below normal. Sixty-five percent of the GH-treated children reached an adult height within the normal range, but the mean final adult height was approximately 10 cm below the genetic target height for boys and 12 cm below the genetic target height for girls. The final height in the control children was 15.8 cm lower than the genetic target in boys and 16.1 cm lower than the genetic target in girls. Although the bone age increased faster during the prepubertal period in the GH-treated children than in the controls, it did not reduce overall height gain. Multiple regression analysis revealed that the absolute as well as the standardized height gain during the observation period was significantly associated with the longer duration of the prepubertal and pubertal observation periods, a longer duration of GH therapy, a greater initial target height deficit, a lower percentage of time spent on dialysis, and male sex. These factors explain 61% to 87% of the variability in the outcome data.

The authors point out that this study provides evidence that GH treatment can sustain catch-up growth in the majority of children with growth failure due to chronic renal failure.

Haffner D, et al. *N Engl J Med* 2000;343(13):923-929.

Editor's comment: *This is a particularly important study because it is the first to look at final height achieved in this population. Clearly, GH therapy is of significant benefit to final height in children with chronic renal failure. The particular strengths of this study are the variety of causes of chronic renal failure in these children and the careful matching of the etiologies between the treated and control groups. An unanswered question is the effect of GH therapy on adult height in children who begin such treatment during their pubertal years. The data in the current study cannot be used to answer this question. The children in this study had glomerular filtration rates of <60 mL/min/m². It also will be important to evaluate the effect of GH therapy on children with lesser degrees of renal insufficiency but similar degrees of growth retardation.*

William L. Clarke, MD

The Impact of Recombinant Human Growth Hormone Treatment During Chronic Renal Insufficiency on Renal Transplant Recipients

Fine et al described the posttransplant outcome for renal transplant patients who were treated with GH therapy during the course of their chronic renal insufficiency. Subjects were identified from 2 control studies (n=194) and matched with patients in the North American Pediatric Renal Transplant Cooperative Study (NAPRTS) database; 95 "likely" matches and 7 "possible" matches were made. These 102 patients formed the GH-treated cohort group and were compared with a control group of 4913 transplant recipients in the database who did not receive GH therapy during their chronic renal insufficiency. Interestingly, the treated cohort tended to have more males, a larger percentage of subjects between 6 and 12 years of age, and more (67% vs 45%) living parent donors.

Two deaths occurred in the cohort, after 78 days and 5 years. The survival rate for the cohort at 3 years was 98.9%, while that for the control group was 95.1%. In the cohort group, 11.8% of grafts failed; 21% of the grafts failed in the control group. There is no statistically significant difference between graft survival rates for either donor source. The percentage of failed grafts with chronic rejection as the cause was marginally significantly higher than in the control group (P=0.05). However, the percentage of all grafts that failed as a result of chronic rejection was similar for the 2 groups (6.9 for the GH-treated cohort and 6.5 for the control).

The mean height Z score at 60 months was slightly improved in the treated group compared with a slight worsening in the control group. In both groups, the delta Z score was positive, indicating continued improvement from baseline. Adverse events in the treated cohort included 2 posttransplant lymphoproliferative disorders and 38 other events, including appendicitis, gastroenteritis, pneumonia, other infections, and hypertensive crisis.

There was no core of adverse events but a broad spectrum of unrelated events. The authors' data did not support the assertion that recombinant human growth hormone (rhGH) treatment during the course of chronic renal insufficiency predisposed to the development of malignancy after transplant.

The authors conclude that GH therapy was not associated with an increase in adverse effects on graft function, nor were there more malignancies posttransplantation. There were concerns that "catch-down" growth would occur after renal transplantation in individuals who received GH during renal insufficiency, which might nullify gains in height. These data do not substantiate these concerns.

Fine R, et al. *J Pediatr* 2000;136(3):376-382.

Editor's comment: *The results are reassuring to physicians treating short children with chronic renal insufficiency with rhGH. Data from this study do not suggest a negative effect of such pretransplant therapy. Mean height scores in the treated group at baseline and 60 months posttransplant were −1.92 and −1.90, as compared with the control group at −1.88 and −2.10. Thus, gains made in height were not lost. This article and an accompanying article by Haffner et al (*N Engl J Med *2000:343[13]:923-930) provide significantly helpful and reassuring information regarding the safety and effectiveness of treating children with rhGH. The reader also is referred to a lead article in* GGH *(Vol. 12, No. 4, p 49) titled, "Recombinant Human Growth Hormone Therapy for Children With Chronic Renal Insufficiency: An Update 1996," which addressed the subject of rhGH treatment in chronic renal insufficency.*

William L. Clarke, MD

Treatment of Acromegaly With Pegvisomant, a Genetically Engineered Human Growth-Hormone Receptor (hGHR) Antagonist

The present investigators report the beneficial effects of a GH receptor (GHR) antagonist in adults with acromegaly. Genetic engineering has permitted development of a mutated GH molecule with replacement of 9 amino acids that increases its affinity for one of the binding sites on the receptor and abolishes binding to the second site, thereby preventing functionally correct dimerization of the receptor. Polyethylene glycol polymers, which are covalently bound to a protein, are stated to be pegylated, thus, the name pegvisomant. Since the GHR is unable to dimerize, signal transduction is inhibited, leading to decreased IGF-I production.

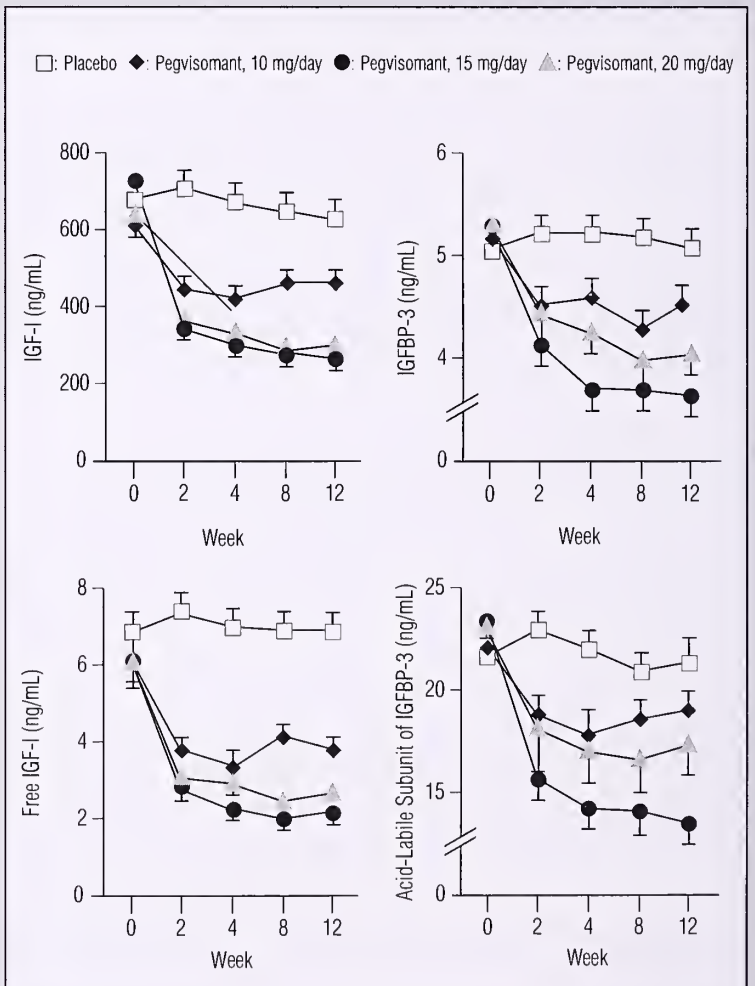
In short-term (12-week) studies, 112 acromegalic adult subjects who had failed previous treatment (surgery and/or radiation and/or dopaminergic agonists, but not long-acting analogues of somatostatin) were divided into 4 groups, including a control group and 3 groups receiving different doses of pegvisomant. IGF-I concentrations (Figure) fell in a dose-dependent manner. Symptoms of GH excess ameliorated as there were significant decreases in soft tissue swelling, diaphoresis, and fatigue. The score for total symptoms and signs of acromegaly decreased significantly in all groups receiving the drug. As expected, serum concentrations of GH increased substantially during treatment in the patients who received 15 or 20 mg of pegvisomant. Anti-GH antibodies were noted in 5 patients but were without physiologic consequence. No patient had a significant change in tumor volume during the study. One patient had alterations in liver function while receiving this agent. No serious adverse effects were otherwise noted. The long-term consequences of the elevated GH concentrations remain to be determined.

Trainer PJ, et al. *N Engl J Med* 2000;342:1171-1177.

Editor's comment: *Neurosurgical removal of GH-secreting pituitary adenomas has been and remains the primary mode of therapy for acromegaly. Medical treatment of hypersomatotropism has been reserved as secondary management; estrogens, dopaminergic agonists (bromocriptine, cabergoline), and short- and long-acting somatostatin analogues (depot preparations of octreotide and lanreotide) that impair GHRH release and inhibit its function at the somatotroph membrane have been employed to lower GH production and decrease IGF-I generation. The introduction of a GHR antagonist has expanded the therapeutic boundaries for this disease, which is so difficult to treat. In another study,*

Figure

Serum Concentrations of Insulin-Like Growth Factor I (IGF-I), Free IGF-I, IGF-Binding Protein 3 (IGFBP-3), and the Acid-Labile Subunit of IGFBP-3 in Patients With Acromegaly.



For all 4 measures, the values at all visits after baseline (week 0) were significantly lower ($P \leq 0.05$) in the 3 pegvisomant groups than in the placebo group. T bars indicate means =SE.

Reprinted with permission from Trainer PJ, et al. *N Engl J Med* 2000;342:1171-1177.

Pegvisomant also lowered IGF-I concentrations and ameliorated symptoms in acromegalic subjects resistant to treatment with octreotide. Whether this GHR antagonist or later generations of GH antagonists will be useful in children is a matter for study. One hopes that such agents will not be employed to alter the growth of normally tall children, but its use in other overgrowth syndromes will be of interest to explore in controlled settings.

Allen W. Root, MD

Herman-Bonert VS, et al. Growth hormone receptor antagonist therapy in acromegalic patients resistant to somatostatin analogs. *J Clin Endocrinol Metab* 2000;85:2958-2961.

Normal Growth Velocity Before Diagnosis of Celiac Disease

Celiac disease has been shown to result in nutritional growth retardation even in asymptomatic patients. However, there are instances in which this disease does not alter normal physical growth.

To evaluate height velocity of patients with confirmed celiac disease before and after diagnosis, anthropometric measurements were taken in 23 patients aged 0.1 to 10.66 years of age. All patients studied during the first 6 months of life showed normal growth velocity, and 6 of 10 patients showed normal growth velocity during the second 6 months of life. Ten of 12 patients between 1 and 2 years of age showed normal growth velocity and 7 of 9 patients aged 2 to 10 years also showed normal height velocity. The authors concluded that celiac disease could be present in children who are growing at a normal rate and that appropriate height and growth should not be factors that exclude the possibility of celiac disease.

Lejarraga H, et al. *J Pediatr Gastrenterol Nutr* 2000;30:552-556.

Editor's comment: *This paper is interesting as patients with confirmed celiac disease were followed longitudinally with reliable anthropometric data. While most of us have stressed the presence of short stature and delayed growth as 2 of the most important clinical manifestations of celiac disease, it is important to be aware of the existence of untreated patients who grow at normal rates. This paper clearly documents that this indeed occurs but is contrary to the usual clinical presentation. Normal growth found in patients with celiac disease requires an explanation. The length of the lesion in the small bowel could be a factor leading to normal or abnormal growth. In countries where the prevalence of celiac disease is high, clinicians should be alerted to the possibility of this disease in a normal, asymptomatic, short-statured child with a previous history of diarrhea or iron deficiency anemia.*

Fima Lifshitz, MD

2nd Editor's comment: *Unfortunately, the authors made only a minimal statement regarding the weight-to-height relationship. Twelve of the 23 patients had normal height and height velocity at diagnosis. Of all the children, 6 also showed normal weight increments before diagnosis. We can only assume that the phenomenon described occurs in children of normal weight for height and in children of low weight for height.*

Robert M. Blizzard, MD

Nutritional Rickets in African-American Breast-Fed Infants

Kreiter and associates report the characteristics of infants and children diagnosed with nutritional rickets at 2 medical centers in North Carolina in the 1990s. Records of 30 children were reviewed; 57% of these presented in 1998 and 1999. All were black and all were breast-fed (average duration of breast-feeding, 12.5 months). Breast-feeding has increased significantly since 1988 (Figure) in North Carolina in both black and white women. Children older than 1 year had a history of poor intake of fortified cow's milk or other dairy products. The age of diagnosis ranged from 5 to 25 months, but one third presented at 12 months of age or younger. Sixty-three percent were diagnosed between April and October, some of the warmer spring/summer months in this southern area. As expected, presenting signs included skeletal abnormalities (n=16) such as bowing of the legs, flaring of the wrist, costochondral beading, fractures, failure to thrive (n=13), hypocalcemic tetany/seizures (n=2), and developmental delay (n=1). Length was <5th percentile in 17 of 26 of the infants (65%), and only 2 patients had a length >50th percentile. With the exception of 1 patient who had recently begun vitamin D treatment, all patients had hypophosphatemia. Sixty percent had hypocalcemia, and 100% had elevations in alkaline phosphatase.

All of the children with rickets were breast-fed without vitamin D supplementation. A survey of 400 pediatricians in North Carolina revealed that 42% prescribed vitamin supplements for all breast-feeding infants, whereas 42% prescribed supplemental vitamins only for selected breast-feeding infants (ie, those with dark skin who are being exclusively breast-fed for more than 4 to 6 months or who are premature). The authors also note that the 1997 American Academy of Pediatric Policy Statement indicates that "vitamin D and iron need to be given before 6 months of age in selected groups of infants (vitamin D for infants whose mothers are vitamin D deficient or those infants not exposed to adequate sunlight)" but that no guidance is given as to how to test mothers for vitamin D deficiency.

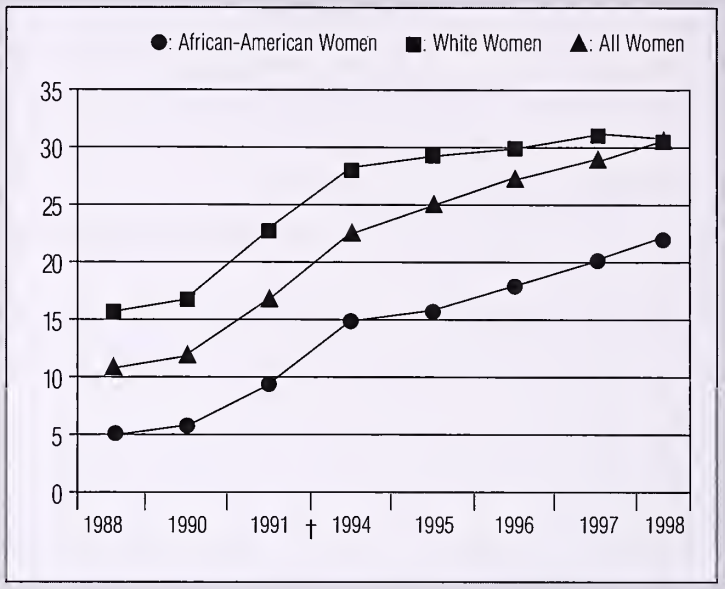
Kreiter S, et al. *J Pediatr* 2000;137(2):153-157.

Editor's comment: *Although this is primarily a descriptive report, the information provided is of significant importance not just to pediatric endocrinologists but to all physicians. In this editor's personal experience, I have seen 2 such children in the past 6 months (1 who was 13 months of age and 1 who was 4 years old). Of interest, the 4 year old was referred for evaluation of short stature and failure to thrive. His lower limb bowing and metaphyseal flaring were obvious at cursory inspection.*

With the significant increase in breast-feeding, accompanied by a significant increase in public health warnings regarding the effects of excessive sunlight and the subsequent use of sunscreen on many infants, it is important that all physicians be aware of the possibilities of vitamin D-deficient rickets and that children be supplemented appropriately. In addition, it is important that the community and physicians be reminded of the signs and symptoms of this easily treatable cause of short stature. A study of subclinical rickets in both white and black infants who are breast-feeding would very possibly determine that the incidence of clinical or subclinical rickets is very significant in the latter group.

William L. Clarke, MD

Figure



Incidence of breast-feeding in African-American women in North Carolina, 1988 to 1998. Information for women seen for the maternal postpartum WIC visit. †Data not available for years 1992 to 1993.

Reprinted with permission from Kreiter S, et al. *J Pediatr* 2000;137(2):153-157.

The Central Melanocortin System Affects the Hypothalamo-Pituitary Thyroid Axis and May Mediate the Effects of Leptin

In the fasted rodent, in the genetically leptin-deficient mouse *(ob/ob)*, and in the genetically leptin-resistant mouse *(db/db)*, there is secondary hypothyroidism. Kim and collaborators hypothesize that leptin may act through the melanocortin system (MS) upon pituitary thyroid-stimulating hormone (TSH) secretion in adult rats. The basis for the hypothesis is that the MS is known to mediate the inhibitory actions of leptin on feeding. αMSH was administered by cannulae into the third intracerebroventricular (ICV) or into the intraparaventricular nucleus (IPVN), which regulate the secretion of pituitary TSH. Also, similarly injected was the *agouti*-related peptide (Agrp), which is an endogenous antagonist of melanocortin 3 and 4 receptors (MCR-3, MCR-4). When activated by αMSH, these receptors inhibit feeding.

In vitro, Agrp significantly decreases plasma TSH concentrations in the *fed* animal when injected into the ICVN or IPVN. In contrast, αMSH increased TSH levels in fasted rats. In vitro, αMSH increased the release of thyrotropin-releasing hormone (TRH) from hypothalamic slices (Figure), an effect blocked by Agrp. In this in vitro system, leptin increased and Agrp blocked the release of αMSH and TRH.

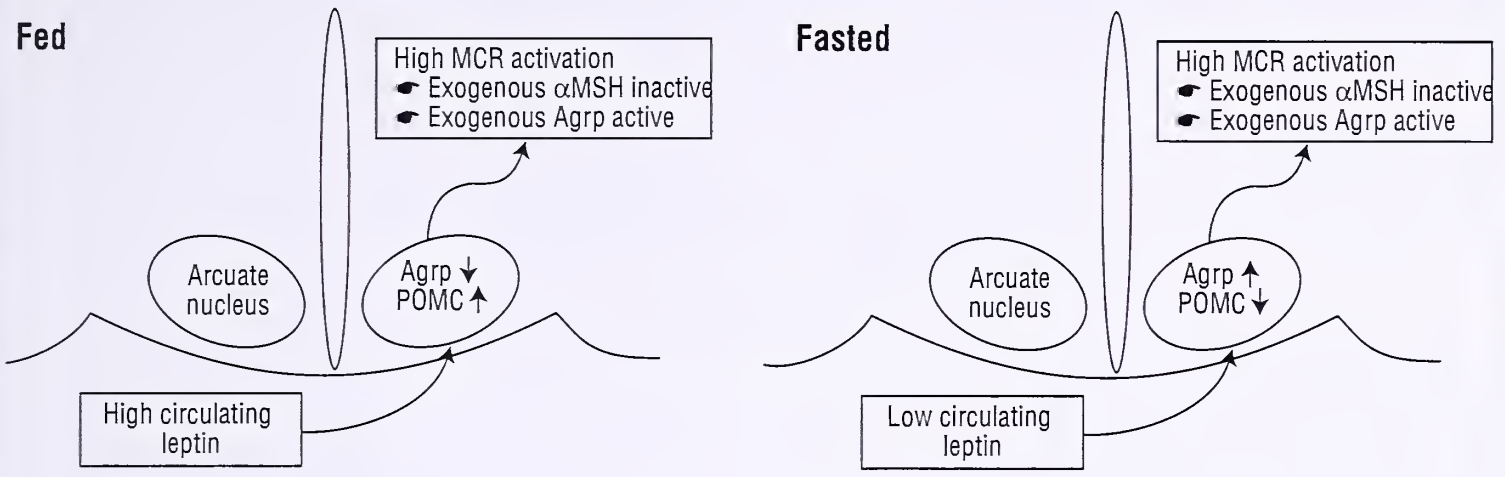
Therefore, the investigators concluded that leptin stimulates thyroid function by enhancing the production of αMSH from pro-opiomelanocortin and possibly by blocking the synthesis of Agrp. αMSH stimulates release of TRH, which increases TSH secretion. Consequently, the regulatory pathways for the control of energy balance via food intake and food metabolism are linked.

Kim MS, et al. *J Clin Invest* 2000;105:1005-1011.

Editor's comment: *In the starved state, the expression of TRH in the paraventricular nucleus is dramatically decreased, a response that can be reversed by the administration of leptin. In addition to the pathway through pro-opiomelanocortin and αMSH synthesized in the arcuate nucleus, leptin likely acts directly on transcription of the gene encoding TRH (Figure).[1] Thus, changes in leptin secretion mediate the metabolic responses characteristic of the fed or starved states. Interestingly, MCR-3 and MCR-4 mediate different aspects of leptin-αMSH actions: MCR-3 affects feed efficiency—that is, the quantity of weight gained per calorie ingested—while MCR-4 influences the quantity of food ingested (or appetite) and energy utilization.[2] Not only does leptin mediate feeding behavior and energy expenditure by its central action, this fat-derived protein also influences bone mass in this manner. ICV administration of leptin inhibits bone formation in* ob/ob *mice by unknown mechanisms, while patients with a loss-of-function mutation in MCR-4 are obese and have a high bone mass.[3]*

Besides its effects on melanin synthesis and dispersal by keratinocytes and on feeding mediated primarily by MCR-4, αMSH, acting through 1 of 5 MCRs, reduces a number of

Figure



POMC = pro-opiomelanocortin

A hypothetical model showing changes of hypothalamic POMC, Agrp, and melanocortin receptor activation in fed animals, fasted animals, and hypothalamic slices. In the fed state, high level of circulating leptin increases POMC and decreases Agrp, resulting in high activation of melanocortin receptor. In this situation, exogenous αMSH is inactive and Agrp is active. In the fasted state and in the hypothalamic slice, low or no circulating leptin induces changes of the central melanocortin system, low POMC, high Agrp, and low melanocortin receptor activations. In this situation, exogenous αMSH is active and Agrp is inactive.

Reprinted with permission from Kreiter S, et al. *J Pediatr* 2000;137(2):153-157.

inflammatory processes by lowering the production of several proinflammatory cytokines (including interleukin-1β and -6, tumor necrosis factor-α, and interferon-γ) and the nuclear transcription factor NF-kB.[4] Serum concentrations of αMSH are elevated in a number of inflammatory illnesses, including HIV infection, suggesting that αMSH may be an important component of our innate host defense mechanisms. Melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide that in fish causes the aggregation of melanin within melanophores and thus lightens the color of the fish scale, an effect opposite to that of and opposed by αMSH.[5] Acting through G-protein coupled receptors, MCH increases food intake in rodents, an effect opposite to that of leptin and αMSH. MCH also acts within the pituitary, where it stimulates corticotropin secretion. Thus, the regulation of feeding and energy metabolism is becoming ever more complex.

William L. Clarke, MD

1. Flier JS, et al. Leptin, nutrition, and the thyroid: the why, the wherefore, and the wiring. *J Clin Invest* 2000;105:859-861.

2. Cummings DE, Schwartz MW. Melanocortins and body weight: a tale of two receptors. *Nat Genet* 2000;26:8-9.

3. Ducy P, et al. The osteoblast: a sophisticated fibroblast under central surveillance. *Science* 2000;289:1501-1504.

4. Catania A, et al. α-Melanocyte-stimulating hormone in normal human physiology and disease states. *Trends Endocrinol Metab* 2000;11:304-308.

5. Saito Y, et al. Melanin-concentrating hormone receptor: an orphan receptor fits the key. *Trends Endocrinol Metab* 2000;11:299-303.

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of this issue. Please follow the instructions listed there to receive CME Category 1 credit. Please note that a question may have more than one correct answer.

1. The adult with TS who has a ring X chromosome is more likely to have more congenital anomalies than one with a 45,X karyotype.
   a. True
   b. False

2. (A) The surviving fraction of TS patients alive at 60 years of age in the study conducted by Price et al was _____.
   a. 52%
   b. 68%
   c. 88%

   (B)The surviving fraction of the general UK population at age 60 years was _____.
   a. 52%
   b. 68%
   c. 88%

3. The areal BMD, which is the usual BMD measured, has been reported to be low in TS patients. However, the volumetric BMD, which is a calculated method, was not reported to be low.
   a. True
   b. False

4. The short stature in TS patients is believed to be related to which *one* of these?
   a. Chondrodystrophy
   b. GH deficiency
   c. Absence of a *SHOX* gene that is located on the sex chromosome
   d. Absence of the *SRY* gene
   e. Autoimmune disease

5. The following diseases are frequently found in TS patients. Which is/are believed to be of autoimmune origin in these patients?
   a. Insulin-dependent diabetes mellitus
   b. Non–insulin-dependent diabetes mellitus
   c. Hypothyroidism

6. (A) In the literature in 1990 a total of _____ pregnancies were reported.
   a. <100
   b. 100 to 150
   c. >150

   (B) Of the pregnancies reported, approximately _____ produced a live neonate.
   a. 40%
   b. 60%
   c. 80%

7. Which of the following statements is/are correct?
   a. Aortic aneurysms occur with and without aortic coarctation.
   b. The increased risk of heart disease and atherosclerosis in TS is consistent with the recent finding from death certificates that approximately 50% of all deaths in TS patients were caused by cardiovascular disease.
   c. TS women should be considered at high risk for coronary thrombosis.
   d. Between 30% to 60% of TS patients have renal anomalies; 6% to 10% of adult TS women may have silent hydronephrosis.

Answer Key: 1.b 2A.b 2B.c 3.a 4.c 5.c 6A.c 6B.b 7.a,b,c,d

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Drs. Ross, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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Androgens in Puberty: Roles in Metabolism and Growth

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INTRODUCTION

Testosterone is the predominant hormone of male puberty, and it greatly impacts the transformation of a boy to an individual with full adult body composition and reproductive maturity. Most of its production comes from the testes in males, but some also comes from the adrenal glands. Androgens also play a role in female reproduction, and excessive production is a common reason for endocrine referral of adolescent girls. Many of the metabolic and growth effects of androgens in normal individuals and in those with altered physiology during this critical period of childhood are reviewed here. For brevity, most of the data reviewed apply to males unless otherwise stated.

PHYSIOLOGY OF ANDROGEN PRODUCTION IN MALE PUBERTY

The prepubertal gonad is relatively quiescent prior to the onset of puberty in terms of sex steroid output. Testosterone concentrations are typically undetectable from about 3 months of age until puberty; however, it is clear that some testosterone must be produced to suppress gonadotropin output. This is evidenced by the marked increase in gonadotropins in patients with anorchism studied in early childhood, suggesting that the prepubertal gonad is active, albeit minimally, substantially before the onset of puberty.

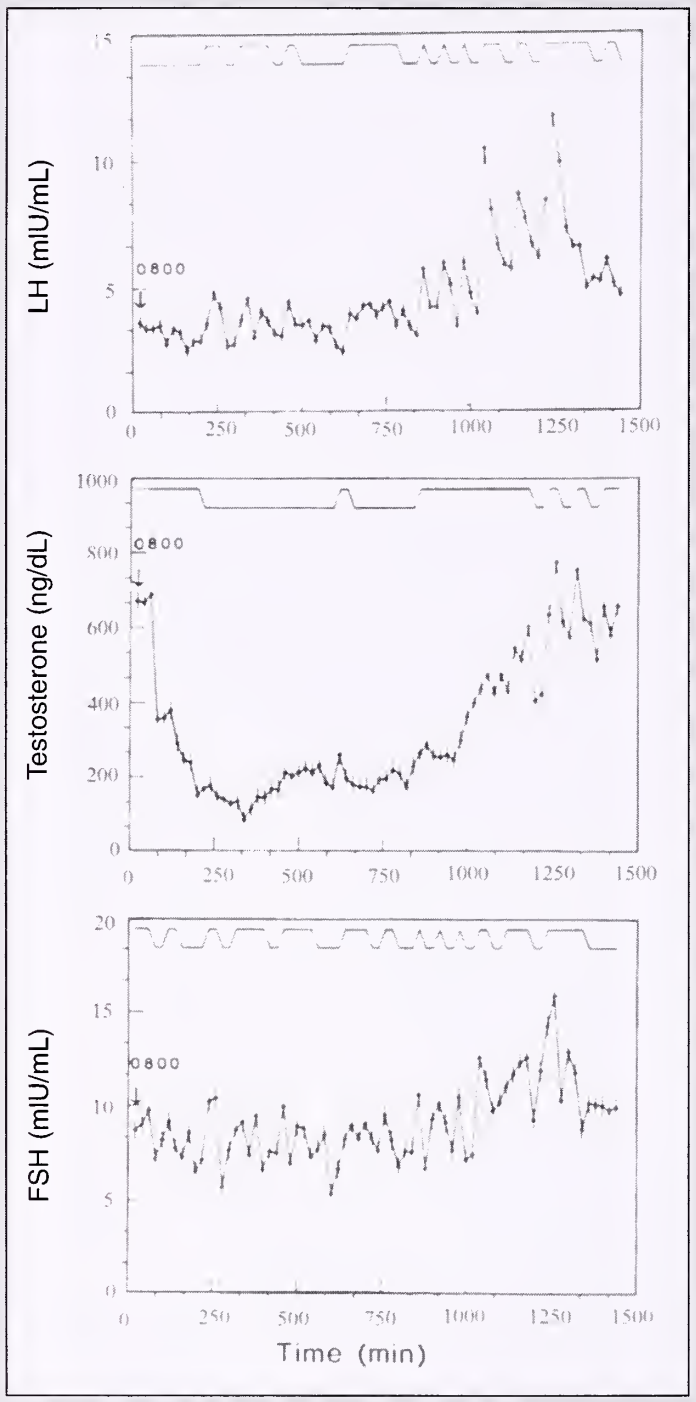
At a mean of 11.2 years, the gonadotropin hormone-releasing hormone (GnRH) pulse generator (gonadostat) increases the amplitude of its pulses, generating increased production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), *particularly at night*. This generates a marked increase in gonadal steroid production, with daily testosterone production rates in mature males of ~5 to 6 mg/d. Testosterone concentrations change rapidly from levels <10 ng/dL in most conventional assays in prepubertal males to concentrations of 350 to 970 ng/dL when the boy reaches Tanner stage V of sexual development. The pattern of testosterone production has both ultradian and circadian rhythms. Minute-to-minute changes in testosterone concentrations can be detected by using frequent sampling methods. Much greater concentrations are found in the early morning, as compared with the afternoon, in normal youngsters studied with blood withdrawn every 20 minutes for 24 hours.[1] These changes parallel the minute-to-minute changes observed in LH and FSH concentrations, which illustrates the functional coupling of these neuronal and hormonal events (Figure 1, page 22). We have observed levels of testosterone in the mid 600 ng/dL range in the early morning in normal boys in late puberty which drops to 50 to 60 ng/dL in the afternoon of the same day.[2] This underscores the need for early morning sampling of random testosterone concentrations when studying the pubertal progress of an adolescent male. What effect, if any, these marked hormonal changes may have on the metabolic effects of testosterone on, or on the typical mood swings of, the teenage boy awaits further study. These changes in testosterone concentrations within the day are not observed in the adult male, who characteristically has relative stability of testosterone concentrations throughout the day.

In This Issue

This educational activity is supported by an unrestricted educational grant from Genentech, Inc.

Figure 1

Luteinizing Hormone (LH), Testosterone, and Follicle-Stimulating Hormone (FSH) Concentration Profiles Measured Every 20 Minutes for 24 hours in 1 Pubertal 14-Year-Old Boy



Note the striking decline in serum testosterone concentrations in the afternoon. Note the deflections at the top of each panel. These represent pulses detected by cluster methods.

Reprinted with permission from Mauras, et al.[1]

Testosterone concentrations markedly impact growth hormone (GH) release in puberty. Comparison of the pulsatile profile of GH concentrations between prepubertal and fully mature (Tanner stages IV and V) boys of similar age reveals that during puberty there is a substantial augmentation (more than doubling) of GH production rates, mostly as an amplitude-modulated phenomenon, that is relatively independent of changes in GH pulse frequency. [2] This rise in GH production is replicated when testosterone is administered to hypogonadal boys[2] but not when nonaromatizable androgens like oxandrolone or dihydrotestosterone (DHT) are administered to similar subjects.[3,4] The impact of testosterone on GH release in puberty is abolished when pubertal boys are given an estrogen receptor blocker (tamoxifen)[5] and is increased when a nonsteroidal androgen receptor blocker (flutamide) is administered, the latter increasing testosterone and hence peripheral aromatization of androgens to estrogen.[6] Taken in aggregate, these data strongly support the concept that the impact of testosterone on GH release is mediated via aromatization to estrogens during puberty.[7]

IN VIVO EFFECTS

Testosterone and its 5α reduced metabolite, DHT, share a common androgen receptor (AR) transcribed from a single-copy gene in the X chromosome. The AR is a member of a superfamily of intranuclear receptors, including receptors for estrogen, progesterone, vitamin D, glucocorticoids, thyroid hormone, and retinoic acid. Testosterone binds to the AR and then activates a number of specific DNA sequences called androgen response elements. These initiate a complex signaling transduction cascade of events that result in the modulation of gene transcription and protein synthesis.

GROWTH

Androgens are critical for a normal and timely pubertal growth spurt in males. This effect is largely mediated by the impact of gonadal steroids on GH production, as discussed above. However, there is evidence for a local effect of androgens on epiphyseal cartilage. Experimental data in human fetal epiphyseal chondrocytes reflect that both testosterone and DHT promote DNA synthesis and that DHT rather than testosterone appears to be the active androgen.[8] Also, using specific monoclonal antibodies against the human AR, experiments demonstrate that there are ARs in human bone in situ, providing evidence for a direct action of androgens on bone and cartilage cells.[9]

Both GH and testosterone appear to be indispensable for normal pubertal growth. This is evidenced by the lack of pubertal growth spurt in children with isolated GH deficiency[10] and by the much slower than normal pubertal growth pace of GH- and testosterone-deficient children who are treated with testosterone but not GH.[11] Even though these observations may be explained by the lack of increase in GH production typically observed in normal puberty, sex steroids probably directly influence the pubertal growth spurt even in GH-deficient states.[12] This apparent synergy of effects of GH and androgens on the pubertal growth spurt has prompted the study of the proper dosing of GH-deficient children in puberty. In a recent

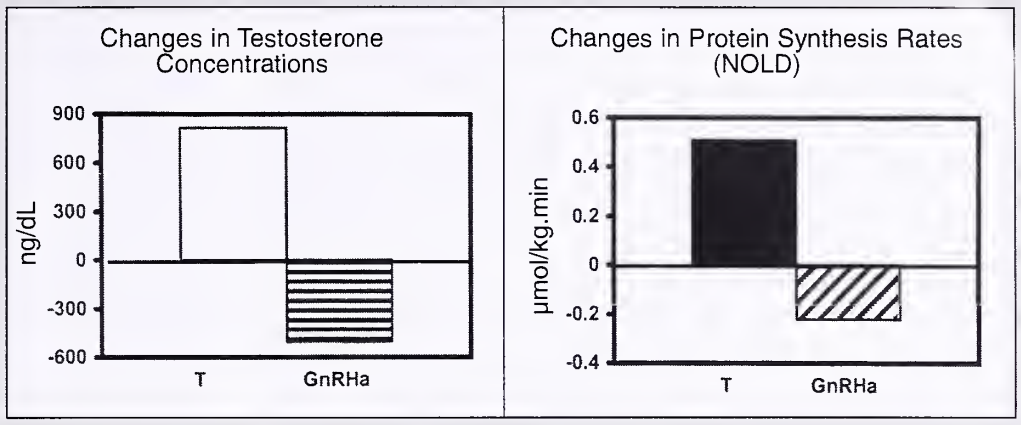
Figure 2

Effects of GnRHa Administration in Young Eugonadal Males

Left panel: Absolute changes in testosterone concentrations after 4 weeks of testosterone treatment in young prepubertal boys, and after induction of hypogonadism with a GnRH analogue in young men studied after 10 weeks.

Right panel: Absolute changes in nonoxidative leucine disposal (NOLD), a measure of whole-body protein synthesis in the same groups of subjects.

Reprinted with permission from Mauras N, et al[15,16]



study, GH-deficient pubertal children treated with high doses of daily GH (0.7 mg/kg/wk) grew taller than those treated with conventional doses (0.3 mg/kg/wk), suggesting that during the narrow window of puberty, higher GH doses may be beneficial, particularly for those suffering the most growth retardation at the start of puberty.[13]

The process of epiphyseal fusion in males has been well characterized. Estrogens are essential in this process and are synthesized by aromatization of androgens, primarily testosterone.[14] Whether timed aromatase blockade in pubertal males will safely increase the height potential of growth-retarded boys in this period awaits further study.

PROTEIN METABOLISM AND SKELETAL MUSCLE

Androgens have potent effects, increasing lean body mass, muscle bulk, and skeletal muscle strength in humans. Using stable isotopes of leucine and glutamine, we have previously shown that testosterone administration to prepubertal males markedly increased whole-body protein turnover and decreased protein oxidation, resulting in a net increase in rates of whole-body protein synthesis.[15] The administration of a GnRH analogue (GnRHa) to eugonadal young men resulted in opposite results, ie, decreased whole-body protein turnover and protein synthesis rates with a marked increase in protein oxidation and decreased lean body mass (Figure 2).[16] The latter was observed despite invariant GH and insulin-like growth factor 1 (IGF-1) concentrations. Testosterone treatment of elderly men, however, is associated with increased mRNA expression of IGF-1 in skeletal muscle,[17] effects opposite of those observed in GnRHa-treated healthy young males, who had decreased mRNA gene expression for IGF-1 after induction of testosterone deficiency.[16] Taken collectively, these data suggest that testosterone per se can affect protein metabolism and body composition, independent of changes in GH production at the systemic level. However, it appears that testosterone is necessary for the normal function of the intramuscular IGF-1 system.

These effects of testosterone are likely direct and not secondary to aromatization, as evidenced by the documented increase in skeletal muscle protein synthesis that occurs after the short-term administration of a nonaromatizable androgen, oxandrolone, to healthy young men.[18] In addition, estrogen therapy does not seem to affect large protein pools at the whole-body level. This deduction is supported by the lack of effect of estrogen administration to hypogonadal girls treated for 4 weeks with oral estrogen[19] and the lack of change in protein synthesis of young males treated with an aromatase inhibitor.[20]

The administration of physiologic or supraphysiologic doses of testosterone has been shown to increase skeletal muscle strength in both elderly and young men,[17,21] and the induction of a hypogonadal state with GnRHa results in a quantifiable loss of muscle strength as measured by isokinetic dynamometry.[16] This effect of testosterone is principally responsible for the marked increase in strength in male puberty. Despite these physiologic effects, the administration of testosterone as an ergogenic agent to young boys is not warranted because of the potentially negative impact of accelerating epiphyseal fusion.

Recently, we compared in prepubertal GH-deficient boys the effects of testosterone administered alone and testosterone and GH administered in combination for 4 weeks, each in random order. We observed a marked increase of the effects of these hormones when given together on IGF-1 production, protein synthesis rates, and body composition, supporting further the concept that these 2 hormones are synergistic in their metabolic effects during puberty.[22]

LIPID AND CARBOHYDRATE METABOLISM

Lipolysis has been shown to occur with androgen stimulation in a variety of experimental situations in both animals and humans. Treatment of rat adipocyte precursor cells with testosterone results in an increase in the number and externalization of ß-adrenergic receptors and increases

forskolin-induced (cyclic adenosine monophosphate [cAMP]-mediated) lipolysis.[23,24] Testosterone also increases triacylglycerol lipase activity.[25] When testosterone is administered to hypophysectomized rats, it does not affect lipolysis. However, when given in conjunction with GH, it normalizes rates of lipolysis in vitro more than GH alone.[26] When young men were rendered hypogonadal by the administration of a GnRHa, we observed marked changes in body composition, with decreased lean body mass and increased adiposity.[16] This was associated with decreased lipid oxidation rates, suggestive of decreased free fatty acid mobilization and substrate availability for oxidation in the absence of testosterone.[16] These and other data support the concept that testosterone and GH have additive effects on lipolysis and help explain the large changes in body composition, increased lean body mass, and decreased adiposity characteristic of male puberty.

During human puberty there is a decrease in insulin sensitivity, as measured by hyperinsulinemic clamp techniques. Puberty is a state of relative insulin resistance.[27,28] Whether these changes are secondary to the increase in GH and/or gonadal steroids is not entirely clear, however. The effects of androgens on glucose metabolism and insulin action have yielded conflicting results. Whereas testosterone treatment of oophorectomized rats resulted in decreased insulin sensitivity,[29] studies in women with polycystic ovary syndrome have shown either an improvement in insulin sensitivity with antiandrogens[30] or no change.[31] We studied adolescent girls with ovarian hyperandrogenism using isotopic infusions of glucose and indirect calorimetry and observed no changes in measures of glucose production or in glucose oxidation rates after normalization of the testosterone levels with oral estrogen/progesterone treatment.[32] These and other data suggest that androgens may not have a critical effect on carbohydrate metabolism. Whether there is a less than critical effect remains a possibility.

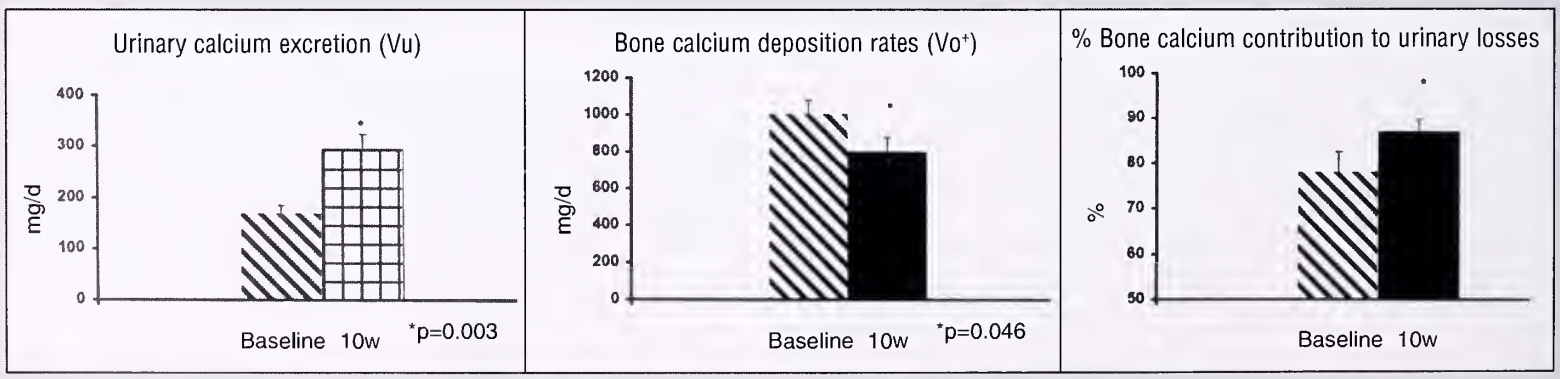
BONE METABOLISM

Androgens are important anabolic agents in bone and are important in bone remodeling. Hypogonadal men have relative osteopenia, and testosterone replacement has marked beneficial effects on bone in males with delayed puberty or with pathologic hypogonadism.[33-35] Androgen deficiency in males induces an initial, rapid increase in bone loss and increased remodeling, followed by a diminished rate of bone formation.[36] We studied bone turnover using stable tracers of calcium in young boys treated with testosterone for 4 weeks and observed significant increases in intestinal calcium absorption and kinetic measures of bone calcium accretion.[15] These changes were opposite to those observed in young males treated with a GnRHa, who experienced marked urinary calcium losses after only 10 weeks of sustained hypogonadism (Figure 3).[37] These effects of testosterone are only partly due to aromatization to estrogen, as supported by several lines of evidence. First, the osteopenia observed after orchiectomy is prevented by the administration of nonaromatizable androgens;[38] second, there is bone loss in female animals given an antiandrogen (flutamide) despite estrogen replacement.[39] In addition, we observed a preservation of bone calcium turnover rates by using an aromatase blocker in young men,[20] contrary to the marked bone calcium loss that was observed after gonadal axis suppression with a GnRHa.

Males continue to actively accrue bone mass even after the completion of linear growth. Peak bone mass in males is not achieved until they are in their mid-20s.[40] Hence, any delay in the timing of sexual maturation can have negative consequences for bone health and potentially increase the risk for osteoporosis.[41,42]

Figure 3

Changes Before and After 10 Weeks of Sustained Hyogonadism In Young Men



Urinary calcium excretion (Vu) (left panel), bone calcium deposition rates (Vo+) (middle panel), and the contribution of bone calcium to the urinary losses (right panel) (n=7).

Reprinted with permission from Mauras N et al.[37]

DISORDERS OF ANDROGEN DEFICIENCY AND EXCESS

The treatment of disorders of androgen production in children requires precise knowledge of the metabolic actions of these steroids and of the dynamic interactions between androgens and GH. Boys with delayed puberty present with sexual infantilism during the teenage years, at the time of the anticipated development of puberty. Low doses of long-acting preparations of testosterone (50 to 100 mg of testosterone enanthate or cypionate, depending on weight) for 6 to 12 months are frequently useful in beginning the process of virilization without negatively impacting skeletal maturation and ultimate height.[43] This also may be achieved with low doses of a nonaromatizable androgen like oxandrolone.[44] Neither testosterone patches nor gels are warranted in this patient group until such a time when the concentrations available for use are low enough in these preparations to prevent premature fusion of the growth plates.

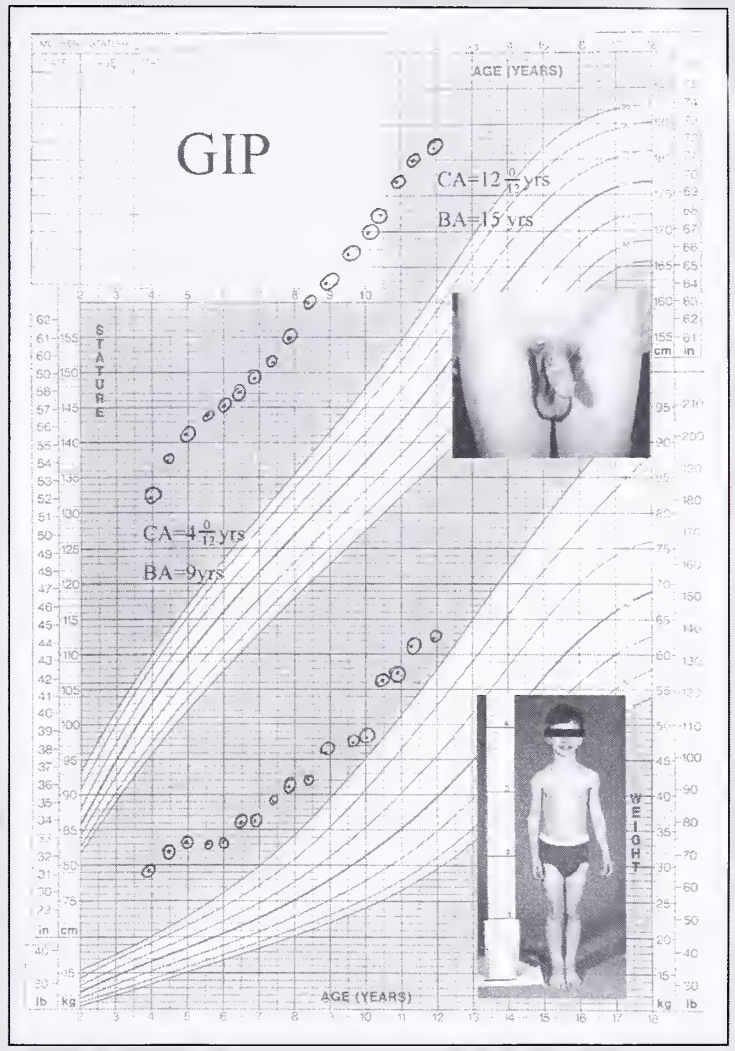
For permanently hypogonadal youngsters who do not have short statures, low doses of testosterone should be used until near adult height is achieved, at which time much higher and fully virilizing doses of androgens should be used, either by injection (depot testosterone, 200 mg every 2 weeks) or via testosterone patches (5 mg/d). The use of testosterone gels in this age group has not been adequately studied to date. Careful assessment of bone mineralization using dual-energy X-ray absorptiometry (DEXA) scanning also can be performed to possible advantage.

Precocious puberty or pseudopuberty in the male, in contrast to girls, is usually secondary to identifiable organic pathology. The differential diagnosis includes adrenogenital syndrome, central precocious puberty due to brain tumor, infiltrative diseases of the brain, cranial irradiation, and CNS trauma. Extracranial germ cell and adrenal tumors also can present with sexual precocity. This is considered when signs of puberty are present prior to age 9 years in boys. The treatment of each of these conditions is disorder-specific; therefore, cortisol supplementation in adrenal hyperplasia, surgical removal or irradiation of brain tumors, and/or excision of extracranial tumors should be considered. When there is premature activation of the hypothalamic-pituitary-gonadal axis, either as a primary cause of the sexual precocity or secondary to the impact of chronic androgens on the hypothalamic gonadostat as in untreated adrenal hyperplasia, treatment with a GnRHa is indicated. In cases of familial male (X-linked) precocious puberty (testotoxicosis), the gonadal activation is independent of gonadotropins and secondary to a constitutive activation of the LH receptor due to a mutation in the LH receptor gene. Attempts to suppress androgen production and androgen effects have proven difficult in this condition. At times striking virilization and temporary tall stature occurs in young boys, and very pronounced bone age advancement occurs (Figure 4), which produces ultimate adult short stature. Treatment with earlier generations of weak androgen receptor blockers (eg, spironolactone) leads to excessive aromatization of circulating androgens, necessitating aromatase blockade (testolactone), thus making treatment cumbersome.[45] Alternative approaches with newer, more potent aromatase inhibitors (eg, Arimidex®) as well as nonsteroidal androgen receptor blockers (eg, flutamide) are being studied. Alternatively, the use of the antifungal ketoconazole, which blocks the cytochrome P450 enzyme involved in androgen synthesis, and the testicular desmolase has been proven useful.[17,20,46] The latter, however, requires careful monitoring of liver function and cortisol production.

Lastly, the treatment of girls with virilization disorders not due to adrenal hyperplasia syndromes can be challenging

Figure 4

Growth Curve



Growth curve for a boy with gonadotropin-independent precocity (GIP), also known as familial male precocious puberty (testotoxicosis). Pictures are at presentation at the age of 4 years. CA, chronologic age; BA, bone age.

Reprinted with permission from N. Mauras, MD

Many times these girls present with premature pubarche, excess body hair, severe acne, and irregular periods. The most common disorder is ovarian hyperandrogenism, in which the ovary is the predominant source of androgens. This is typically, although not always, associated with obesity and hyperinsulinemia. The role of insulin in the pathogenesis of this disease has only begun to be unraveled.[47] Suppression of excess androgens by putting the ovary "at rest" with the use of birth control pills is effective in controlling excess androgens, and is commonly combined with an antiandrogen, most commonly spironolactone or more recently flutamide. Newer strategies such as suppression of the cytochrome P450 enzyme with metformin[48] or insulin-sensitizing agents like rosiglitazone, typically used to treat type 2 diabetes mellitus, are offering additional choices for the treatment of these virilized girls.[49]

SUMMARY

This brief review of a variety of in vitro and in vivo studies permits one to conclude that androgens are critical for the normal development of puberty in males. Androgens potentiate linear growth, mostly indirectly via enhancement of GH's production by way of aromatization to estrogens and, to a lesser extent, directly via the effects on the growth plate. Testosterone potently stimulates whole-body and muscle protein synthesis, improves skeletal muscle strength, and facilitates lipolysis. Yet it has less important effects on carbohydrate metabolism. Androgens appear to be critical for bone health in males. Treatment of children with androgens in deficiency states and the use of GnRH analogues and antiandrogens in males, and suppression of androgen production in females, requires careful assessment of the endocrine mechanisms operative in the given disease state and a thorough understanding of the metabolic actions of these potent anabolic hormones in childhood.

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

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Height Outcome in Congenital Adrenal Hyperplasia Caused by 21-OH Deficiency: A Meta-Analysis

There are reports in the literature of significant short final height associated with viralizing congenital adrenal hyperplasia (CAH). These final heights, which average –2 standard deviations (SD) or lower, are not related to the dose of glucocorticoid, degree of hormonal control achieved, or age at initiation of therapy. Eugster and colleagues review their experience regarding adult height in 65 of their CAH patients over a 20-year period. To be included in their study, children had to be 5 years of age or older. Age at diagnosis, target height, and adult final height were examined. Early diagnosis was defined as a diagnosis made at less than 1 year of age. Actual and predicted height values were expressed as SD scores, and compliance was assessed by querying physicians. For patients who had not yet reached adult height, predictions of adult height were derived using the child's most recent bone age. Of the 65 patients whose charts were examined, 23 had completed their linear growth, and compliance was judged to be good in 28. The overall mean final height SD score minus the target height SD score was –1.03 (–4.21 to –2.32). There was no difference seen between males and females. However, a trend (not statistically significant) for better height outcome was seen in patients with good compliance. Those patients identified as having been diagnosed early tended to have a better final height minus target height SD score than those identified later (again, not statistically significant).

In addition, a Medline database search was conducted of all publications reporting height outcome in CAH patients. The meta-analysis identified 16 studies with data that could be used to provide similar outcome information. The current study was added to those data (see Figure). The mean weighted final height SD score for all studies was –1.7; in the subset of studies in which target height could be determined, the final height minus target height SD score was –1.21. In this larger group, a statistically significant difference was seen between patients who were diagnosed early versus late.

The authors state that their data, as well as that from the meta-analysis, demonstrate that adult stature within 1 SD of genetic target may be achieved by many CAH patients with the use of traditional therapy. Also, those subjects diagnosed at an early age have a significantly better outcome than those identified later, and compliance appears to confer some advantage in final height. They correctly point out the flaws in their analyses, including the retrospective design, the prediction of final height in two thirds of the subjects as opposed to actual measurements, and the subjective evaluation of compliance. They stress, however, that rather than pursuing alternative therapies for CAH, efforts should be focused on early detection and improved compliance.

Eugster EA, et al. *J Pediatrics* 2001;138:26-32.

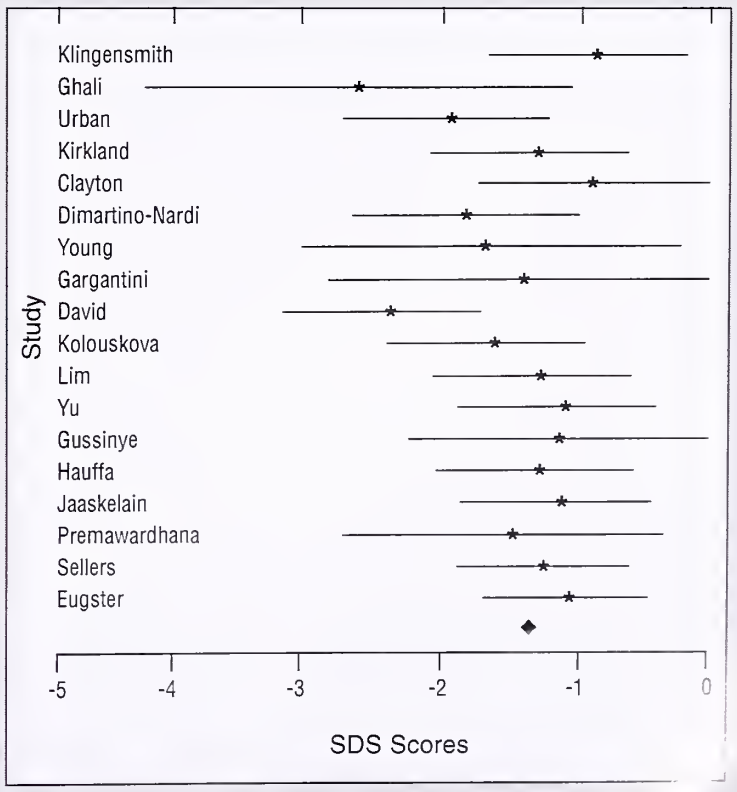
Editor's comment: *The information provided by this study should be of considerable interest to all pediatric endocrinologists. The flaws in the data, as emphasized by the authors, are significant, even though the results of their particular study are similar to those in the literature. Presenting information regarding final height when that height is a predicted value for more than two thirds of the subjects makes the results highly speculative. Indeed, those subjects who are still growing—6 of whom were teenagers—may have had changes in their compliance that later could have affected their adult height. Closing the door on alternative therapy suggests that some physicians may be better at securing compliance in their patients. A previous article in* GGH *(2001;15(3):33-41) reviews the adult consequences of CAH. This review, which included 5 of the studies listed in the current article, showed that nearly all subjects were shorter than expected, with little influence of age at diagnosis on outcome. Although these data should not be construed as refuting the conclusions provided by Eugster et al, nonetheless there is considerable variability in outcome in different clinics and, presumably, among different patient groups. Indeed, decreased adult height has been correlated with increased body weight and body mass index (BMI) during childhood, suggesting that those children who may be overtreated in attempts to suppress androgen production may have a significant risk of reduction in final height. Clearly, those patients could have benefited from alternative treatments.*

William L. Clarke, MD

2nd Editor's comment: *Achievement of optimal growth in children with CAH is a well-recognized challenge in the treatment of this disorder. Often there is an inability to adequately suppress*

Figure

Overall Mean SD Score of Final Height for Each Study in Meta-Analysis With 95% CI



Solid diamond indicates weighted mean SD score for all studi... ...so demonstrated is lack of correlation between year of pub... ...n and outcome.

Reprinted with permission from Eugster EA, et al. *J P... ...trics* 2001; 138:26-32.

corticotropin stimulation without simultaneously incurring the deleterious effect on growth of glucocorticoid overtreatment. This study clearly points out that adult stature in most children with CAH is within 1 SD of the genetic target, with at least one third of the patients achieving their target height. This study reassures pediatric endocrinologists that adequate treatment of patients diagnosed early might lead to achievement of an adult height appropriate for the family. However, there might be opportunities for advances in clinical management combined with diagnostic precision by the molecular genetic characterization of these patients, ie, the CYP21 *gene. The heterogeneity of the disease and/or the concept that all patients with CAH need treatment with mineralocorticoid replacement, regardless of their salt-wasting status, needs to be considered to improve the outcome. However, the most practical item is for us to devise ways to improve compliance with the treatment over prolonged periods. For example, it was recently shown that treatment with dexamethasone in a convenient once-a-day dosage may be easier for the patients and yet allow them to achieve a normal growth (see the next abstract for details).*

Fima Lifshitz, MD

3rd Editor's comment: *The reader's attention is redirected to Dr. Clarke's comments above pertaining to BMI, increased body weight, and adult height. After rereading, proceed to an abstract in this issue entitled, "Body Mass Index in Childhood and Its Association With Height Gain, Timing of Puberty, and Final Height."*

Robert M. Blizzard, MD

Dexamethasone Treatment of Virilizing Congenital Adrenal Hyperplasia (VCAH): The Ability to Achieve Normal Growth

The authors summarize their 2 decades of experience with the long-term, routine use of dexamethasone (DEX) in the treatment of children with 21-hydroxylase- and 11-hydroxylase-deficient CAH (N=26 [23 with salt loss] and 5, respectively). Administration of DEX began as early as birth and continued for an average of 7 to 8 years and for as long as 20 years. DEX elixir was administered once daily (0.1 mg/mL) at a mean dose of 0.27 mg/m^2/d (range, 0.24 to 0.33 mg/m^2/d). Fludrocortisone was given as needed. The hypothalamic-pituitary-adrenal axis was effectively suppressed with this regimen.

In the 19 subjects whose bone age was within 2 years of chronologic age at the initiation of DEX, there were comparable increases in chronologic, height, and bone ages in both males and females. Achieved or predicted adult heights were similar to estimated target heights (see Figure).

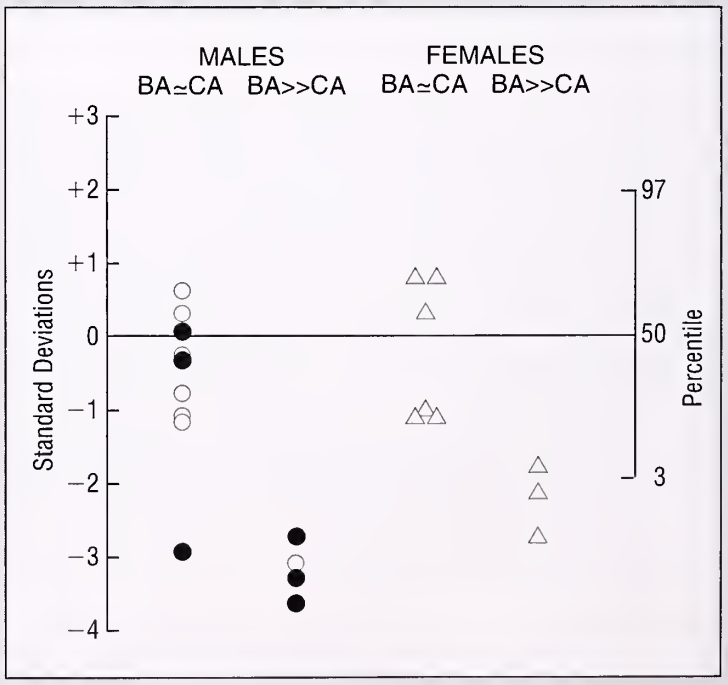
In 7 children in whom bone ages were more than 2 years in advance of chronologic age when treatment with DEX was begun, linear growth relative to advancement in bone age improved but did not achieve unity. The authors conclude that DEX is an effective and safe glucocorticoid for the management of CAH in childhood.

Rivkees SA, Crawford JD. *Pediatrics* 2000;106:767-773.

Editor's comment: *Management of infants and children with CAH remains a challenging task primarily because of the need for rigid adherence to the usual therapeutic program—particularly the administration of cortisol at close to 8-hour intervals. While achievable in infancy and early childhood, strict compliance becomes more difficult as the patients' schooling and other activities increase. Thus, the report by Drs. Rivkees and Crawford is welcome and useful. Many of us have been reluctant to utilize DEX in infants and children with CAH, although it is effective in older adolescents and young adults, because of its evident biopotency. Based on their experience, the authors calculated that 1 mg of DEX is 70-fold more effective than 1 mg of cortisol in suppressing adrenal function, rather than the 30-fold potency stated by the manufacturers. As the authors point out, a comparison of the effects of DEX to those of another group of children with CAH treated more conventionally would have been useful. It would seem reasonable to undertake such a comparative long-term trial, if possible. Assuming these data are confirmed, DEX would seem preferable to the use of androgen receptor blockers and aromatase inhibitors in the management of children with CAH in order to keep treatment as uncomplicated as possible.*

Allen W. Root, MD

Figure
Mature and Predicted Adult Heights After Treatment With Dexamethasone



Mature heights (solid symbols) or predicted adult heights (open symbols) after treatment with dexamethasone. Heights are given as SDS or percentile. △, females; ○, males; BA, bone age; CA, chronologic age.

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GROWTH, Genetics, & Hormones
Volume 17, Number 2

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7. a b

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_____

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Celiac Disease in Children and Adolescents With Type I Diabetes: Importance of Hypoglycemia

This article explores the association of celiac disease and type 1 diabetes mellitus in a retrospective case-controlled study. Patients with type 1 diabetes mellitus were screened for celiac disease by measurements of both serum immunoglobulin (Ig)A antiendomysial (EMA) and antigliadin (AGA) antibody levels. The diagnosis of celiac disease was confirmed by small-bowel biopsy when testing for EMA and/or AGA antibodies was positive. Patients were matched for age, sex, and duration of disease for the 18 months before and after the diagnosis of celiac disease. Metabolic control was assessed by hemoglobin A_{1c}, frequency of hypoglycemia, and total insulin requirements for the 18 months before and after the diagnosis of celiac disease.

There were 20 patients of 434 with type 1 diabetes who had celiac disease. None of them had symptoms or signs typical of this disease. However, during the 6 months before and after diagnosis of celiac disease, these patients had more hypoglycemic episodes than the controls: 4.5 vs 2 severe episodes with a progressive reduction in insulin requirement of 0.6 vs 0.9 µ/kg/d. The introduction of a gluten-free diet led to normalization of the intestinal mucosa and reduced the frequency of hypoglycemia in the celiac disease patients. The prevalence of celiac disease in this population of type 1 diabetes mellitus was 4.6%. All 414 control patients had negative tests for EMA and AGA antibodies. The authors concluded that underlying celiac disease should be suspected in patients with diabetes mellitus presenting with symptomatic hypoglycemia.

Editor's comment: *The association between celiac disease and type 1 diabetes has long been known. The coexistence of these 2 entities appears to be due to a common genetic predisposition attributed to the presence of the locus human leukocyte antigen (HLA) DR3. This report, as well as other studies using serologic data, describe a celiac disease prevalence of 5% to 7% in patients with type 1 diabetes mellitus. Often these patients do not present with any symptoms of overt malabsorption. However, as the authors point out, the occurrence of hypoglycemia in a child with diabetes mellitus should lead to screening for celiac disease. Measurements of EMA or AGA antibodies should be obtained, and, if positive, a confirmatory small-bowel biopsy should be performed even in patients who appear to be asymptomatic. These patients may have malabsorption of a sufficient degree to interfere with carbohydrate absorption with a resultant increased risk for hypoglycemia. It should be kept in mind that the prevalence of celiac disease in normal children might be about 1%* (Pediatrics *2001;107:42-45), whereas in type 1 diabetes patients the prevalence is at least 4 times higher. Thus, we should proactively consider routine screening for this disease in type 1 diabetes patients, just as we screen for other diseases (eg, hypothyroidism).*

Fima Lifshitz, MD

Mohn A, et al. *J Pediatr Gastroenterol Nutr* 2001;32:37-40.

Obesity, Increased Linear Growth, and Risk of Type I Diabetes in Children

Hypponen and associates report for the Childhood Diabetes Study Group in Finland on their evaluation of the effect of obesity and linear growth on the risk of developing type 1 diabetes during childhood. All children under the age of 15 years who had type 1 diabetes diagnosed between September 1986 and September 1989 were invited to participate in the study. All the study participants were tested for antibodies associated with diabetes. Ninety-eight percent were found to be positive for at least 1 type of antibody, confirming that they had autoimmune type 1 diabetes. Age- and sex-matched nondiabetic control children were randomly selected from the Finnish National Population Registry. Neonatal data and sociodemographic data were collected using structured questionnaires. An equal proportion of the diabetic and control children lived in rural areas. Information regarding height and weight was obtained from well baby clinics and school healthcare units for the 586 children with diabetes and for the 571 controls. Heights were available for both parents for the majority of study subjects. Relative weight calculated as "weight in relation to mean weight for height" and relative height as "a deviation of height in SD scores" were computed using the Finnish growth standards. Statistical analysis was based on relative weight and relative height in relation to age. Three age groups were studied: 2 weeks to 1.9 years, 2 to 9.9 years, and 10 years and older.

Neither the mean relative weight nor the relative height at birth differed between the diabetic and control subjects. But both boys and girls who developed type 1 diabetes weighed more than the control children from infancy onward. There was a significant difference between the diabetic and control boys with regard to relative height from early infancy on. Among the girls, this significant difference was present until 10 years of age. Unfortunately, there were only limited data available for girls after the age of 10 years. Adjustments for neonatal and sociodemographic characteristics, or target heights, did not affect the results of this study. Both *higher relative weight* and *greater relative height* were associated with an increased risk of developing type 1 diabetes, and the magnitude of the effect was somewhat greater with respect to relative weight in infancy and early childhood. The effect of relative height remained constant throughout all ages.

The authors remind us that obesity is a well-known risk factor for type 2 diabetes, and that obesity is an increasing problem in many countries. In Finland, the annual incidence of type 1 diabetes has increased more than 4 times between 1953 and 1998. The role of obesity in this increase is unclear. Unequivocally, the increase in risk of type 1 diabetes for 1 SDS increment in relative height was 20% to 30%. Obesity or relative weight >120% after 3 years of age was associated with a more than 2-fold risk of developing type 1 diabetes. It is known that there is an association between obesity, accelerated height gain, insulin resistance, or enhanced insulin secretion, and signficant subsequent enhanced insulin secretion. Hyperinsulinemia is obviously associated with

active beta cells, and active beta cells have been shown to be more susceptible to cytokine-induced damage than resting cells in vitro.

Hypponen E, et al. *Diabetes Care* 2000;23:1755-1760.

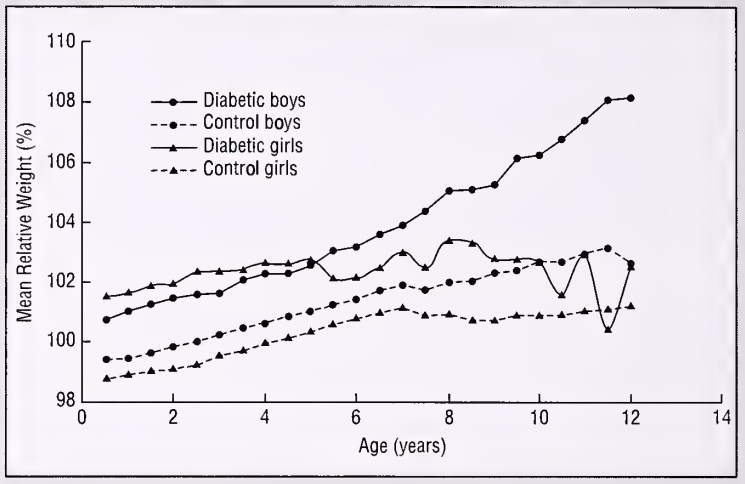
Editor's comment: *This is a very interesting and important article. The incidence of type 1 diabetes in Finland is exceedingly high, much higher than that in the United States. The association of early childhood obesity and increases in relative height with an increased incidence of type 1 diabetes is significant information and a warning to the pediatric community. Recent reports have documented a significant increase in the incidence of type 2 diabetes among children and adolescents, paralleling the increase in obesity in this group. Hypponen et al's paper is the first to show that an increase in weight also is associated with an increase in type 1 diabetes. The information regarding tall stature is not new, but is consistent with other reports from Europe and the United States.*

The Childhood Diabetes Study Group in Finland has presented information that needs to be transmitted to all physicians caring for children. The prevention of childhood obesity may be one of the most important therapeutic activities of pediatricians.

William L. Clarke, MD

Figure

Cross-Sectioned Mean Relative Weights for Diabetic and Control Groups, Calculated from the Interpolated Values



Reprinted with permission from Hypponen E, et al. *Diabetes Care* 2000; 23;1755-1760.

Neonatal Outcome After Preimplantation Genetic Diagnosis by Analysis of the Polar Bodies

New reproductive technologies have increased the options available to couples. Preimplantation genetic diagnosis (PGD) was developed for couples at high genetic risk to avoid establishing pregnancies with genetic diseases. PGD is performed by blastomere biopsy or polar body removal (PBR) for mendelian or chromosomal disorders. Mothers who are heterozygotes for a mutation are good candidates for this procedure. Primordial germ cells will contain 1 chromosome carrying the affected allele and another carrying a normal allele. During meiosis, the oocyte will double its genetic material, yielding 2 chromosomes with normal alleles and 2 that contain the mutant allele. At the conclusion of meiosis I, the oocyte extrudes half of its chromosomes in the form of the first polar body. When the first polar body is removed before fertilization, it can be analyzed for the presence of the normal or mutant allele. Subsequently, fertilization occurs, the oocyte completes a second meiotic division, and then the second polar body is extruded containing 1 set of chromosomes. The second polar body also can be analyzed, and it will usually be identical to the 1 that remains in the egg. If a crossover occurs during meiosis, the first polar body may contain both mutated and normal alleles, in which case it will be necessary to analyze the second polar body to see which allele will be left in the fertilized egg. It is therefore possible to identify embryos developing from oocytes that contain a normal allele and then to transfer the fertilized oocyte back to the mother and establish a pregnancy.

The present study is the follow-up of the first 97 pregnancies that yielded 109 live-births after PGD by PBR and assessment. Ninety-one infants were born where analysis had been done for chromosomal disorders, and 18 infants were born where analysis had been done for mendelian disorders (including cystic fibrosis, sickle cell disease, long-chain acyl-CoA dehydrogenase deficiency, and thalassemia). All case analyses also were done postnatally to confirm the prenatal diagnosis. Birth data are available for 98% of the cohort, and developmental assessments are available for 44 children older than 6 months of age (see Table, page 31).

There were 80 singleton pregnancies, 9 twins, and 7 triplets, of which 3 were reduced to twins. One gestation with 5 fetuses

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miscarried in the first trimester. There was an increased occurrence of prematurity, and 1 neonate died as a result of placental abruption. The mean singleton birth weight was at the 47th percentile, and the mean singleton birth length was at the 57th percentile. Forty percent of births were by cesarean section, which is comparable to other in vitro fertilization (IVF) studies. There were 6 infants with birth defects: 1 with a unilateral transverse limb reduction (amniotic band syndrome); 1 with neonatal seizures who had 3 cerebral infarcts on imaging; 1 with a minor hemangioma; 1 with minor strawberry hemangiomas on both arms; 1 with thickening of the tricuspid valve that did not require surgery; and 1 with bilateral webbed toes. Only 1 child of the 44 who had been followed up to 6 months of age was reported to have developmental delay. This was 1 of twins who had had no perinatal complications. This child had speech delay and was receiving speech therapy. This frequency of birth defects is certainly not out of line of what would be expected.

The financial cost of PGD by PBR is reported to be $8500 for 1 typical cycle. Diagnostic testing for mendelian traits may involve as much as another $1000 in laboratory costs.

The authors point out that in addition to the financial costs, there are some intrinsic risks of multiple gestations and the complications associated with them. Nevertheless, polar body prenatal diagnosis does provide families with another option in terms of prenatal diagnosis.

Strom CM, et al. *Pediatrics* 2000;106:650-653.

Table
Summary of Preimplantation Genetics Pregnancies

Number of Fetuses	Number of Pregnancies	Number of Spontaneous Abortions	Number of Live Births
1	80	5	75
2	9	1	16
3	7	0	18*
5	1	1	0
Total	97	7	109

*Three couples had reduction to twins; 4 couples delivered triplets.

Reprinted with permission from Strom CM, et al. *Pediatrics* 2000;106:650-653

Editor's comment: *The data from this large center are reassuring. The reliability of testing for mendelian disorders needs further study since there are really only 18 cases. The procedure certainly allows individuals to obtain a diagnosis before implantation, if that fits with their particular ethical stance. Clearly, the cost is much higher than that associated for prenatal diagnosis which is performed later in pregnancy. However, it does not involve termination of pregnancy, and only those embryos which do not have a detectable abnormal test would be used for implantation. The reader may wish to extend his/her knowledge of this alternative diagnostic technique as it undoubtedly will become a common tool of IVF.*

Judith G. Hall, OC, MD

Spectrum of the Tricho-Rhino-Phalangeal Syndromes

Three types of tricho-rhino-phalangeal syndrome (TRPS) have been clinically defined. The features characterizing these syndromes, but described initially in TRPS I, include sparse, slowly growing scalp hair; sparse eyebrows laterally; bulbous tip of the nose; protruding ears; brachydactyly and mild to moderate short stature; and the presence of cone-shaped epiphyses of the middle phalanges on X-ray films. TRPS II is distinguished from TRPS I by the occurrence of exostoses; mental retardation often is present. TRPS III is distinguished by the greater severity of the characteristics of TRPS I.

Mutations of a gene designated *TRPS1,* which encodes a zinc finger transcription factor, were recently identified in patients with TRPS I. Microdeletions of chromosome 8q24.1 that include both *TRPS1* and *EXT1*, the gene mutated in hereditary multiple exostoses type I, are responsible for TRPS II. The current study by Lüdecke et al was done to determine if TRPS III is due to *TRPS1* mutations, representing the severe end of a clinical spectrum of TRPS I, or, alternatively, results from mutations of another gene. The results confirmed the former possibility and demonstrated a correlation between the type of mutation and the severity of clinical phenotype.

TRPS1 was screened by direct sequencing of the coding and flanking intron sequences for mutations in 79 patients with TRPS, including 57 unrelated individuals with either TRPS I or TRPS III. Thirty-five different mutations were found in 44 of 51 unrelated patients. The majority were deletions or disruptions, nonsense and splicing mutations. These would be expected to truncate the transcription factor protein, leading to loss of function, since the resulting proteins would lack a nuclear localization signal needed for nuclear entry and the C-terminal zinc finger domain required for dimerization. These mutations would, therefore, act through haploinsufficiency. Missense mutations were identified in 8 cases. They all mapped to exon 6, which encodes the GATA zinc finger domain necessary for DNA binding. The resulting proteins would be expected to enter the nucleus and form complexes with other transcription factors that would function poorly because of defective DNA binding. They are predicted to exert a dominant negative effect, which as a disease-causing mechanism generally has a greater impact than haploinsufficiency.

The patients also were evaluated clinically, mainly in terms of height and severity of brachydactyly as judged from hand X-rays films. The results showed a continuous spectrum of severity. They further revealed that nonsense and disruption mutations, which would be predicted to cause haploinsufficiency of *TRPS1,* were associated with the range of severity typical of TRPS I. In contrast, the missense mutations predicted to act in a dominant negative fashion correlated with the severe end of the spectrum characteristic of TRPS III.

Thus, *TRPS1* mutations account for TRPS. Loss of function of 1 *TRPS1* allele gives rise to mild to moderate manifestations associated with the diagnosis of TRPS I. Missense mutations that act in a dominant negative manner account for the severe features observed in TRPS III. Chromosomal deletions that cause haploinsufficiency of *TRPS1, EXT1*, and potentially other neighboring genes are responsible for TRPS II.

Lüdecke H-J, et al. *Am J Hum Genet* 2001;68:81-91.

Editor's comment: *This study nicely demonstrates how different types of mutations of the same gene can produce clinical phenotypes that appear to be different. The authors acknowledge that no mutations were detected in a few patients, making it possible that 1 or more other genes could harbor mutations that lead to a TRPS clinical phenotype. However, their conclusion that* TRPS1 *is the major, if not only, gene locus responsible for this constellation of features cannot be disputed. It will be interesting to learn the function of* TRPS1 *in skeletal growth and maturation.*

William A. Horton, MD

BMI in Childhood and Its Association With Height Gain, Timing of Puberty, and Final Height

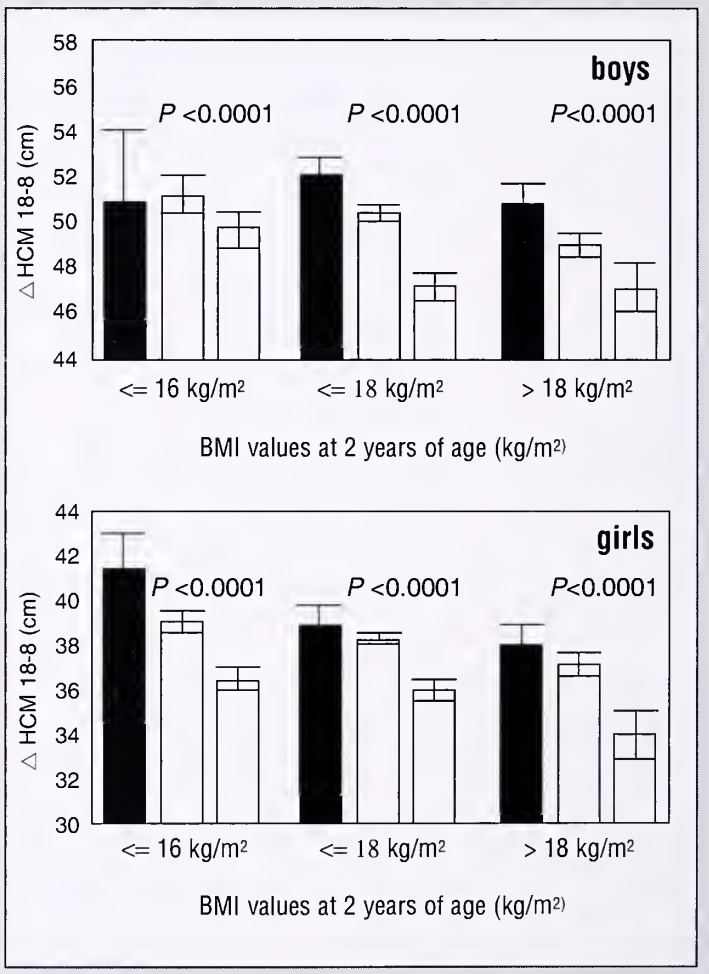
This study was undertaken to ascertain the effects of overnutrition in childhood on height, final height, and timing of puberty. This study was performed in 5111 grade-school children born in the early 1970s in Goteborg, Sweden. The final analysis was made in 3650 full-term healthy children whose growth information was accurate from birth to 18 years of age. The others were eliminated from the final analysis due to a variety of factors and/or illnesses. A computer-generated growth chart was produced for each child, and their nutritional status was assessed by body mass index (BMI) changes between 2 and 8 years of age. Mean parental heights were adjusted to assess genetic influences of the linear growth. Childhood BMI gain was related to an increased height gain during the same period (ie, an increase of 1 BMI unit was associated with an excess increase in height gain of 0.23 cm in boys and 0.29 cm in girls). The BMI also was linked to an earlier onset of puberty; the impact on the timing of puberty was 0.6 years in boys and 0.7 years in girls. Each increased unit of BMI gain in childhood also reduced the height gain in adolescence by 0.88 cm for boys and 0.51 cm for girls. However, no direct effect was found between childhood BMI gain and final adult height. The authors conclude that overnutrition between 2 and 8 years of age may lead to earlier onset of puberty and earlier achievement of adult height, but not greater height.

He Q, Karlberg J. *Pediatr Res* 2001;49:244-251.

Editor's comment: *Overnutrition and/or obesity in childhood is a worldwide health concern because it may produce several adverse physical and psychosocial developmental consequences. Moreover, the obese child is at a higher risk of remaining obese throughout adulthood. Several studies have shown that overnutrition accelerates linear growth. This large population study certainly adds support to this concept. However, postnatal linear growth is complex, resulting from genetic, nutritional, and endocrine system influences. The BMI does not necessarily represent the only variable affecting growth, nor does it represent the true nutritional status of an individual. The effect of dietary attempts to lose weight was not investigated in this study. Usually, children who are obese tend to be on and off diets. This may lead to poor nutrition and potential growth deceleration. However, it is reassuring to know that this large population of obese children did not experience a reduction in final adult height.*

Fima Lifshitz, MD

Figure
The Mean and Its 95% Confidence Interval of △HCM 18-8 for Boys and Girls in 3 Different Groups of BMI Values at 2 Years of Age



The cut off points, 16 and 18 kg/m², represent the 25th and 75th centile values at 2 years of age. Within each BMI group at 2 years, the values of height gain between 8 and 18 years are also shown separately in 3 childhood BMI change groups. The *P* values refer to the ANOVA to compare the differences in central tendency of height gain among the 3 BMI change groups.

Reprinted with permission from He Q, Karlberg J. *Pediatr Res* 2001;49:244-251.

Genetic Ablation of Parathyroid Glands Reveals Another Source of Parathyroid Hormone

Glial cells missing-2 (*Gcm2*) is 1 of 2 mouse homologues of *Drosophila Gcm*, a gene encoding a transcription factor that is involved in the differentiation of neural cells. In *Drosophila*, loss of *Gcm* leads to decreased numbers of glial cells and their presumptive conversion into neurons, while its overexpression increases the number of glial cells. In the mouse, *Gcm2* is expressed only in the parathyroid gland. From embryonic stem cells, Gunther et al knocked out *Gcm2* and created mice heterozygous for loss of this gene by injecting the altered DNA of these stem cells into blastocysts; the trait could then be transmitted as a germline mutation. The heterozygous mice ($Gcm2^{+/-}$) were phenotypically normal.

Mice homozygous for loss of *Gcm2* ($Gcm2^{-/-}$) were produced by mating of heterozygous animals. Because of marked hypocalcemia (3.0 mg/dL), 30% of the homozygous animals died within 8 hours after birth; however, 70% survived with subnormal serum calcium levels of 6 to 7 mg/dL. The surviving $Gcm2^{-/-}$ animals were viable and fertile despite hypocalcemia. They had bone abnormalities consistent with an increase in bone volume such as with hypoparathyroidism. Absent parathyroid glands were determined by histologic examination, and lack of parathyroid hormone (PTH) expressing cells in the thyroid and surrounding tissue. These hypocalcemic rodents had serum levels of immunoreactive PTH, which in an assay that did not cross-react with PTH-rP, were comparable to those in wild-type mice with serum calcium values of 10 mg/dL. However, these PTH levels were too low to restore eucalcemia. No other structural abnormalities were present in the $Gcm2^{-/-}$ mice.

Search of multiple tissues revealed expression of *PTH* in the hypothalamus (confirming a 1990 report) and in a small group of cells in the subcapsular region of the thymus. This small group of cells colocalized with expression of *Gcm1*, the second mouse homologue. DNA sequencing confirmed that thymic *PTH* was identical to that in the parathyroid glands. It could be downregulated by administration of calcitriol but could not be upregulated by further lowering of serum calcium concentrations (by infusion of phosphate). The investigators suggested that thymic PTH secretion was maximal following ablation of the parathyroid glands.

Gunther T, et al. *Nature* 2000;406:199-203.

Editor's comment: *The identification of* Gcm2 *as an essential factor for differentiation of the parathyroid gland in mice suggests that its human homologue (*GCM2, *chromosome 6p24.2, OMIM 603716) may have the same function. The identification of a patient or of a family with this genetic mutation may be anticipated. The affected members of the family would join the gain-of-function mutation in* CASR *(encoding the membrane calcium sensing receptor) and the loss-of-function mutations in* PTH *and* UFDIL *(which is the mutation in the DiGeorge syndrome) as documented familial forms of hypoparathyroidism. The relationship between neural cell differentiation in insects and parathyroid gland development in mammals is intriguing and unexplained at present. That the thymus and the immune system are secondary sources of PTH synthesis and secretion is consistent with their production of other peptide hormones (corticotropin, GH, etc).*

Allen W. Root, MD

Balling R, Erben RG. *Nature Med* 2000;6:860-861.

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University of Virginia, The Blake Center
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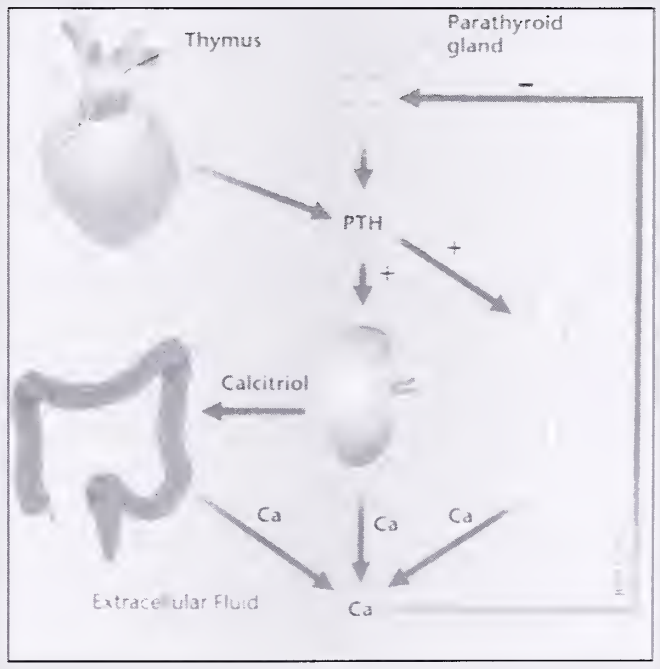
Charlottesville, VA 22903

Figure

Endocrine Control of Calcium Homeostasis



Parathyroid hormone (PTH) is secreted from the parathyroid glands (four circles). A new auxiliary source of PTH has been located in the thymus. PTH increases mobilization of calcium (Ca) from bone by enhancing bone turnover. In the kidney, PTH stimulates tubular reabsorption of Ca and favors the synthesis of the steroid vitamin D hormone, calcitriol. The main physiologic function of calcitriol is to increase intestinal Ca absorption. Therefore, all effects of PTH act to directly or indirectly increase the calcium concentration in the extracellular fluids. An increase in the concentration of ionized Ca in the extracellular fluids is the major feedback mechanism that inhibits PTH secretion from the parathyroid glands and possibly also from the thymus by a Ca-sensing receptor expressed in the membrane of PTH-secreting cells. In the absence of parathyroid glands, thymic PTH secretion seems to be a backup mechanism for emergency regulation of Ca metabolism.

Reprinted with permission from Balling R, Erben RG. *Nature Med* 2000;6:860-861.

Ghrelin: A Gastrointestinal and Hypothalamic Peptide Affecting Hormone Secretion and Fat Metabolism

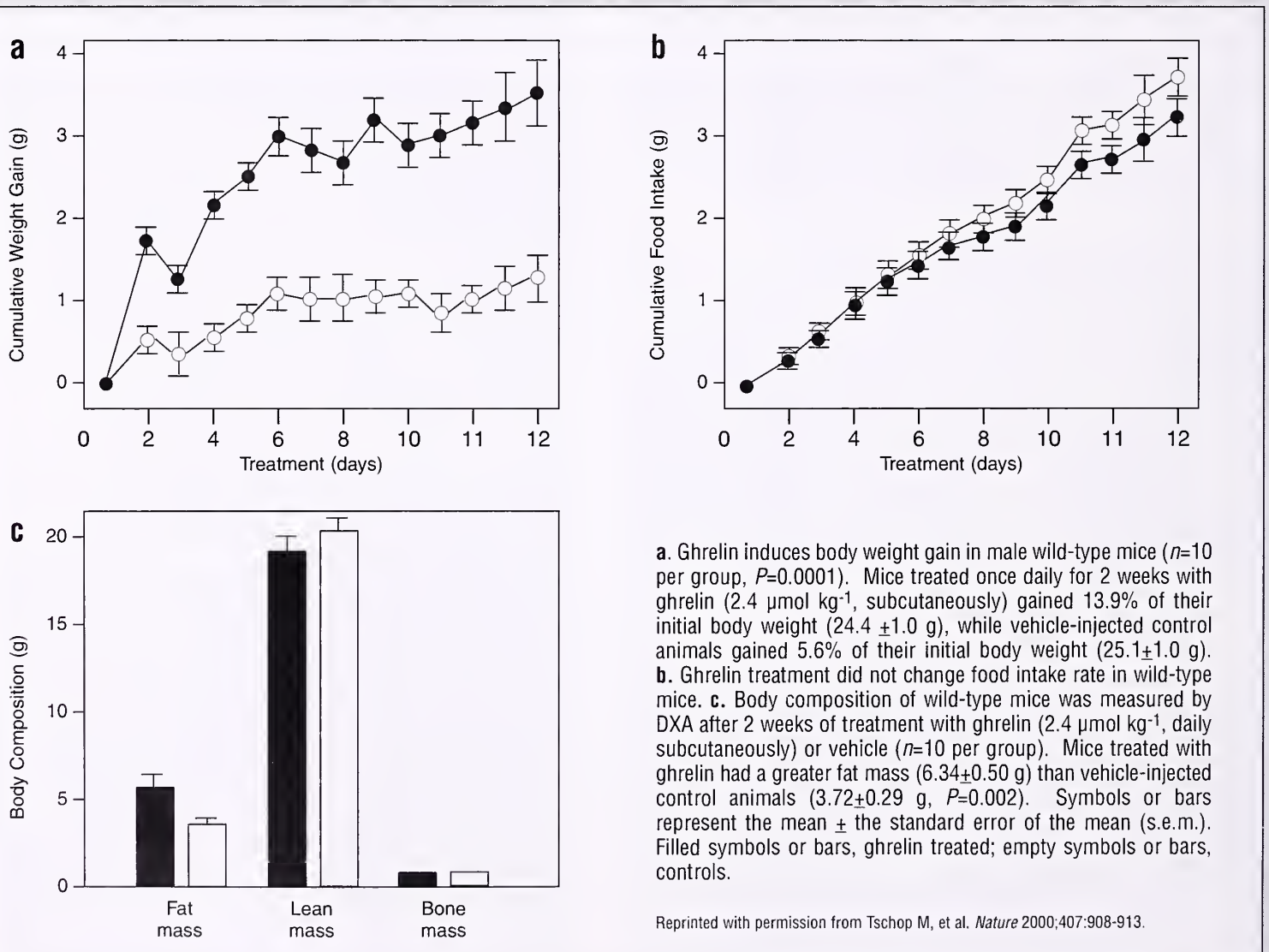
Ghrelin, a 28 amino acid peptide synthesized by the gastrointestinal tracts and hypothalamic arcuate nuclei of rodents and humans, is the natural ligand for the GH secretagogue receptor (GHS-R). Since synthetic GH secretagogues increase weight in experimental animals, both groups of investigators studied the effect of ghrelin on feeding and weight gain in intact adult male rats.

Tschop et al demonstrate that once-daily *subcutaneous administration* of synthetic rat ghrelin (2.4 µmol/kg/d; MW 3313.85) for 14 days doubled the rate of weight gain without inducing hyperphagia and increasing food intake; the increase in weight was due to accumulation of fat without alteration in lean body mass or bone density (see Figure). The isolated increase in fat was related to an increase in respiratory quotient (RQ) of ghrelin-treated rats, indicating that this peptide stimulated carbohydrate utilization while decreasing the rate of fat utilization. Ghrelin did not increase the rate of energy expenditure or the motor activity of recipients. The mechanism by which ghrelin enhanced fat accumulation was not due to its GH-releasing effects as GH was lipolytic in control animals and was similarly effective in GH-deficient dwarf rats and wild-type animals. Its effect was not mediated by the orexigenic neuropeptide Y (NPY), as ghrelin stimulated fat accumulation in *NPY*$^{-/-}$ animals. *Continuous intracerebroventricular (ICV) infusion* of ghrelin for 7 days enhanced weight gain in wild-type rats and increased their RQ and food intake. Tschop et al also observed that fasting increased and feeding decreased serum concentrations of ghrelin in these animals.

Wren et al report that *intraperitoneal administration* of ghrelin (3, 10, and 30 nmol) *acutely* increased food intake only in the

Figure

Ghrelin-Stimulated Adiposity in Mice



a. Ghrelin induces body weight gain in male wild-type mice (n=10 per group, P=0.0001). Mice treated once daily for 2 weeks with ghrelin (2.4 µmol kg^{-1}, subcutaneously) gained 13.9% of their initial body weight (24.4 ±1.0 g), while vehicle-injected control animals gained 5.6% of their initial body weight (25.1±1.0 g). **b.** Ghrelin treatment did not change food intake rate in wild-type mice. **c.** Body composition of wild-type mice was measured by DXA after 2 weeks of treatment with ghrelin (2.4 µmol kg^{-1}, daily subcutaneously) or vehicle (n=10 per group). Mice treated with ghrelin had a greater fat mass (6.34±0.50 g) than vehicle-injected control animals (3.72±0.29 g, P=0.002). Symbols or bars represent the mean ± the standard error of the mean (s.e.m.). Filled symbols or bars, ghrelin treated; empty symbols or bars, controls.

first hour after injection; its *ICV injection* (0.3, 1.0, and 3.0 nmol) acutely increased food intake, with maximum intake in the first hour after injection but with a duration of effect of 24 hours. *ICV* ghrelin increased serum concentrations of GH and corticotropin and decreased those of thyrotropin.

Tschop M, et al. Ghrelin induces adiposity in rodents. *Nature* 2000;407:908-913.

Wren AM, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000;141:4325-4328.

Editor's comment: *Ghrelin is now added to the complex of neuroendocrine and transcription factors that affect feeding behavior and energy metabolism, including peroxisome-proliferator-activated receptor-γ2, leptin, NPY, melanin-concentrating hormone, pro-opiomelanocortin and melanocortin, the agouti protein, and so forth, and provides another site at which weight-control pharmacologic therapeutics may be targeted. The mechanism by which ghrelin selectively spares fat metabolism, thus increasing its accumulation, is unknown at present. Increased RQ without an increase in energy intake might be due to decreased activity of the sympathetic nervous system or to hypothalamic stimulation. The bioeffects of ghrelin are uniquely suited to enhance the anabolic effects of GH, which is maximally effective in the well-nourished recipient.*

Date et al have identified the rat and human gastrointestinal X/A-like cell of the oxyntic gland as the site of synthesis of ghrelin; these cells are located primarily in the fundus of the stomach. Apparently, more than 18 cell types that synthesize endocrine-like hormones have been identified to date in the gastrointestinal tract. There are 4 distinct endocrine cells, each synthesizing a specific product in the oxyntic mucosa of the rat: ECL–histamine; D–somatostatin; enterochromaffin–serotonin; and X/A-like– ghrelin.

Allen W. Root, MD

Date Y, et al. Ghrelin, a novel growth hormone-releasing acetylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000;141:4255-4261.

Kojima M, et al. Ghrelin is a novel growth hormone-acetylated peptide from stomach. *Nature* 1999;402:656-660.

2nd Editor's comment: *A third article, by Nakazato et al in (*Nature *2001;409:194) supplements the above. Rats were injected with ghrelin in the cerebral ventricles, which produced significantly greater weight gain than was observed in controls infused with saline. The authors demonstrated that the increased eating observed was not related to GH secretion. However, ghrelin stimulates not only food intake but also GH secretion. These mechanisms are not interdependent. However, in the normal creature with the capability to respond in a dual manner to ghrelin, the growth action of GH may be enhanced by the increased food ingestion. These early reports are not necessarily synchronous. Confirming and additonal studies are needed and undoubtedly clarification will occur.*

Robert M. Blizzard, MD

Autosomal Dominant Hypophosphataemic Rickets Is Associated With Mutations in *FGF23*

Clinical and biochemical manifestations of autosomal dominant hypophosphatemic rickets (ADHR) are same as to those of X-linked hypophosphatemic rickets (XHR), ie, deformities of the lower extremities, short stature, rickets, hypophosphatemia. XHR has been attributed to loss-of-function mutations in *PHEX*, a gene encoding an endopeptidase that may serve to activate or degrade an as yet uncharacterized protein involved in phosphate transport termed "phosphatonin."

Studies of families with multiple members affected with ADHR by collaborating investigators of the ADHR Consortium linked this disorder to chromosome 12p13.3. Utilizing publicly available genomic sequences from chromosome 12p13, the authors found 37 genes in this region, 13 of which were previously unrecognized. With more discriminating linkage analysis, a segment of chromosome 12p13.3 encoding 11 genes was identified; screening of these genes for mutations revealed 1 with homology to those encoding the fibroblast growth factor (FGF) family that was mutated in patients with ADHR. Previously undescribed, *FGF23* has 3 exons with 1612 bp encoding a peptide with 251 amino acids that has a similar 3-dimensional configuration and 25% to 36% homology with other members of the FGF family; *FGF23* is the largest FGF described to date. In subjects with ADHR, mutations in *FGF23* that segregated with the disease include: NT 527G→A → Arg176Gln; NT 535C→T → Arg179Trp; and NT 536G→A → Arg179Gln.

These changes were not polymorphisms. No mutations of *FGF23* were detected in patients with hypophosphatemic bone disease or in subjects with apparent XHR with normal *PHEX* analyses. In normal human tissues, *FGF23* was expressed predominantly in heart, liver, and thyroid/parathyroid tissue. The physiologic function of *FGF23* was not identified in this report. The investigators speculate that it might be related to or perhaps even be the elusive phosphaturic substance "phosphatonin."

ADHR Consortium. *Nat Genet* 2000;26:345-348.

Editor's comment: *This work illustrates the treasure trove of genetic data already available from the Human Genome Project waiting to be mined for relevance to human physiology and pathophysiology.* PHEX *is expressed by osteoblasts, and it has been hypothesized that "phosphatonin" also may be synthesized by these cells. In normal mouse embryos, the murine homologue* Fgf23 *maps to chromosome 6. The present investigators were unable to demonstrate expression of* Fgf23 *in the tibiae of embryonic mice, perhaps suggesting that* FGF23 *is not "phosphatonin."*

Allen W. Root, MD

Ecarot B, Desbarats M. 1,25-$(OH)_2D_3$ down-regulates expression of *PHEX*, a marker of the mature osteoblast. *Endocrinology* 1999;140:1192-1198.

Post-Program Self-Assessment/CME Verification

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Follow the instructions listed there to receive CME Category 1 credit.

1. Gonadotropins are suppressed by testosterone in childhood.
   a. True
   b. False

2. Testosterone and growth hormone each have a direct effect on bone growth in the early pubertal boy.
   a. True
   b. False

3. A growth spurt at adolescence does *not* occur in the hypogonadal male.
   a. True
   b. False

4. Testosterone and its 5" reduced metabolite, dihydrotestosterone, share an androgen receptor which is transcribed from a single-copy gene in the Y chromosome.
   a. True
   b. False

5. Aromatization of an androgenic steroid means that it is converted, at least in part, to an estrogenic steroid, usually estradiol.
   a. True
   b. False

6. Testosterone given to prepubertal males markedly inhibits whole body protein turnover.
   a. True
   b. False

7. The marked increase in strength in males at puberty is due to the increased production of:
   a. Growth hormone
   b. Testosterone

Answer Key:
1.a, 2.a, 3.a, 4.b, 5.a, 6.b, 7.b

CME Accreditation Statement

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Drs. Mauras, Lifshitz, Clark, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Inc.'s National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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Endocrine Complications of the Successful Treatment of Neoplastic Diseases in Childhood

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INTRODUCTION

Cancers are relatively rare in children and adolescents, with approximately 12,500 individuals younger than 20 years of age diagnosed with a new malignancy yearly.[1] The most prevalent cancers observed before 20 years of age include leukemias (25%), of which most are acute lymphoblastic leukemia (ALL); tumors of the central nervous system (CNS;17%), lymphomas, including Hodgkin's disease (15%); and tumors of bone and soft tissue (13%). The remaining 30% are of other origin.

Mortality rates in the past 25 years have decreased dramatically in contrast to incident rates, which have increased slightly or remained steady. The current 5-year overall survival rate for childhood cancers exceeds 70%, and survival rates currently are 80% for children with ALL and greater than 90% for children and adolescents diagnosed with Hodgkin's disease.[1]

The remarkable improvements in survival result from advances in supportive care such as prevention and treatment of infections; the improved utilization of blood and blood products; and, most importantly, changes in therapy occurring over the past 30 years. Included are the use of combined modality therapies such as the use of surgery with chemotherapy and radiation therapy and the use of aggressive multiagent chemotherapeutic regimens. From our experience at Memorial Sloan-Kettering Cancer Center, as well as the experience of others, approximately two thirds of pediatric cancer survivors will develop medical complication or disabilities attributed to their previous cancer treatment.[2] Endocrine disturbances have been documented in 20% to 50% of survivors and frequently occur as late effects of cancer therapy. An overview of the endocrine complications that develop following successful treatment of childhood cancer will be presented. Emphasis is placed on the observations at the Memorial Sloan-Kettering Cancer Center and from those reported in the literature during the past several years.

GROWTH FAILURE

Impaired linear growth with resultant adult short stature occurs frequently in survivors of childhood cancer, particularly in individuals treated at a young age. The incidence is greater in females than in males. A variety of factors, including high-dose radiation therapy, particularly to the brain and the spinal cord, early pubertal development, hypothyroidism, and growth hormone deficiency (GHD), contribute to the short stature in adult survivors. As is apparent, both nonendocrine and endocrine factors can contribute to growth retardation.

The *nonendocrine factors* affecting growth are primarily intensive oral or parenteral chemotherapy and irradiation of skeletal structures. The administration of chemotherapy is often associated with mild to moderate reduced growth, which in many instances is only temporary. However, the adverse effects on growth can persist more long term.[3] The deleterious effects on growth are dependent on the number and dosage of the drugs and

In This Issue

This educational activity is supported by an unrestricted educational grant from Genentech, Inc.

the duration of treatment, all of which reflect the intensity of the regimen. Glucocorticoids, mercaptopurine, and methotrexate are specific drugs implicated in the inhibition of normal growth. While the mechanism(s) of chemotherapy-induced growth failure remain uncertain, the data suggest that chemotherapy may act both directly on bone growth by suppressing osteoblast and osteoclast activity and through alterations of the growth hormone–insulin-like growth factor 1 (GH–IGF-1) system.[4,5]

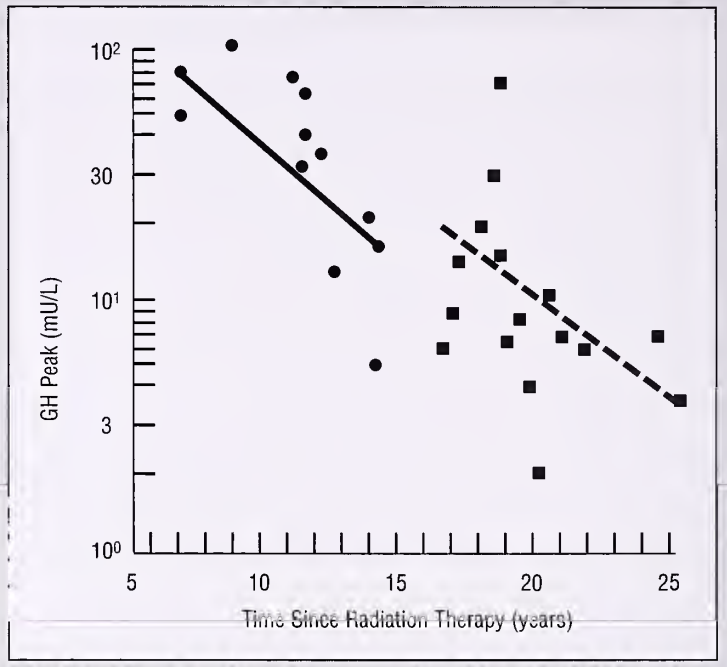
Direct external beam radiation to the spine and, to a lesser degree, to the long bones can produce profound losses in growth potential in children. The ultimate impact on final height depends on the dose of radiation therapy, the volume irradiated, and the age of the subject at the time of treatment. The height reduction that occurs following contemporary radiation regimens for the treatment of diseases such as Hodgkin's disease[6] and Wilms' tumor,[7] where direct external beam radiation to the spine and bones is not intense, is generally quite modest and usually not clinically important.

The *endocrine factors* that disrupt the normal pattern of growth in survivors include GHD and premature sexual development (PSD). Both of these neuroendocrine disturbances usually are the consequence of hypothalamic-pituitary irradiation. Primary hypothyroidism also may contribute to poor linear growth in these children and will be discussed in the section on thyroid abnormalities.

Tumors such as germinomas and optic nerve gliomas, which arise in or near the region of the hypothalamus and pituitary, produce GHD as a direct result of the tumor or as a consequence of the surgery required to remove the tumor. More frequently, however, GHD is diagnosed after exposure of the hypothalamus or, less commonly, after exposure of the pituitary to high-dose, external beam radiation therapy. GHD is most often seen following whole brain irradiation for acute leukemia or for a variety of CNS tumors and after localized radiation therapy for sarcomas and carcinomas of the orbit, face, and nasopharynx. Additionally, GHD does occur following total body irradiation, which is used as preparative therapy for bone marrow/stem cell transplantation.[3,8,9]

Radiation therapy is followed by GHD in both a dose- and time-dependent relationship. External beam radiation doses >30 Gy typically produce GHD within 5 years of treatment; after lower doses, such as 18 to 24 Gy, GHD may not become evident for 10 or more years (Figure 1).[10] Once established, however, radiation-induced GHD is usually permanent. Establishing a diagnosis of GHD can be problematic in this population. First, neither plasma concentrations of IGF-1 or IGF-binding protein-3 (IGFBP-3) appear to be reliable indicators of the GH status following cranial irradiation and, thus, cannot be recommended as screening tests for the presence of GHD in irradiated subjects.[11] Second, the standard provocative tests to release GH can produce false-negative results (ie, normal GH levels despite low spontaneous secretion of GH). Differentiation from normal requires the use of 12- to 24-hour frequent sampling

Figure 1



The fitted lines are from the regression of log 10 of GH peak to an insulin tolerance test versus time since cranial irradiation. Closed circles and solid line, 18 Gy; closed squares and dotted line, 24/25 Gy.

Reprinted with permission from Brennan BMD, et al. [10]

studies, particularly in subjects treated with doses <30 Gy. False-negative results, however, appear to be less common if one utilizes the insulin tolerance test.[12]

Final height is most affected in individuals who are diagnosed with cancer at a young age and who are treated with high doses (>30 Gy) or who receive radiation to the whole brain and/or spine. Additionally, there is some evidence that suggests that females do worse than males, presumably because girls are more likely than boys to enter puberty at an early age following hypothalamic irradiation. Over the past several years, it has become evident that cranial irradiation at both lower and higher doses (35 to 50 Gy) is associated with the development of precocious puberty.[13,14] Age of onset of puberty is directly correlated with age at treatment but indirectly correlated with body mass index. While earlier studies suggested that the tempo of puberty also is accelerated in these patients, recent data have been unable to confirm this. The vast majority of patients with early onset of puberty also will suffer from GHD. Clinical signs of GHD may be obscured by the seemingly normal rate of growth these children manifest, owing to the inappropriate production of sex steroids. However, when viewed within the context of their prepubertal status and bone age, these children usually are found to be growing at a suboptimal rate. It is very important that physicians following these children keep in mind the possibility of these phenomena existing and obscuring GHD.

GH improves the growth rate of children who develop GHD following cancer therapy, at least in the short term. Data accumulated several years ago suggested that most patients, however, achieved a final height significantly below their target height. The poor response

to GH therapy has been attributed both to patient factors such as spinal irradiation, early pubertal onset, and variables in treatment such as suboptimal dosing schedules and to the older age of most patients when started on GH. Recent data suggest that improvements in growth and final height can be achieved with contemporary dosing regimens.[15] Moreover, the addition of a gonadotropin-releasing hormone (GnRH) agonist to suppress puberty in individuals who have sexual precocity may augment final height, but this is based on data derived solely from retrospective, uncontrolled studies.[16] GH-releasing hormone (GHRH) therapy also may improve growth in subjects with radiation-induced GHD, but the data are quite limited.

Concerns over the safety of GH therapy relate to the fact that GH is a potent growth-promoting agent with mitogenic and proliferating properties. However, large-scale studies assessing the risk of tumor recurrence in brain tumor survivors treated with GH have now been reported. All have consistently reported no increased risk associated with GH replacement therapy.[17,18] Because of the paucity of data, uncertainty remains about the risk of disease recurrence when GH therapy is administered to survivors of pediatric cancers other than brain tumors. Similarly, little information is available about the effect of GH replacement on the risk of developing secondary neoplasms in pediatric cancer survivors. The risk of developing slipped epiphyses may be increased in cancer survivors, particularly survivors of leukemia, who were treated with GH compared with children treated with GH for idiopathic GHD.

Young adult survivors with either childhood- or adult-onset GHD, such as that following low-dose cranial irradiation at a young age, also may benefit from GH therapy, especially if they manifest any of the metabolic derangements such as increased body fat, raised plasma lipids, and decreased bone density and/or quality-of-life issues that have come to be recognized as the adult GHD syndrome.

To date there are no studies, however, that have addressed the risks and benefits associated with long-term GH therapy in adult survivors of childhood cancer.

HYPOTHALAMIC-PITUITARY DYSFUNCTION

A variety of neuroendocrine abnormalities result from external radiation to the whole brain, orbit, face, or nasopharynx, with resultant pathophysiology in the hypothalamic-pituitary axis. The larger the dose of radiation and the longer the time interval since completion of therapy, the greater the likelihood of developing any of the given problems. In the majority of instances, the site of damage appears to be at the hypothalamus rather than the pituitary gland. Early puberty and GHD are the most common neuroendocrine disturbances. The threshold dose necessary to induce these problems appears to be about 18 Gy when given in conventional daily fractions.

Clinically evident deficits of luteinizing hormone/follicle-stimulating hormone (LH/FSH), thyrotropin (TSH), and corticotropin occur less often than GHD, and generally only following doses of radiation in the range of 30 to 40 Gy.[19] Deficits of these hormones usually occur several years following irradiation. GHD ordinarily is the first recognizable hormonal deficiency. Interpretation of the available literature about these trophic hormones is complicated by the fact that different investigators employ different hormonal tests and use varying criteria for what constitutes "abnormal." For example, Rose et al[20] report a very high incidence of "hidden" central hypothyroidism secondary to TSH deficiency following cranial irradiation. According to the authors, the establishment of a diagnosis of TSH deficiency often requires performing both a thyrotropin-releasing hormone stimulation test and an assessment of the nocturnal TSH surge. These tests involve obtaining multiple blood samples during the day and night. At present it is unclear whether this subtle form of TSH dysfunction correlates with any clinical findings and, thus, whether

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

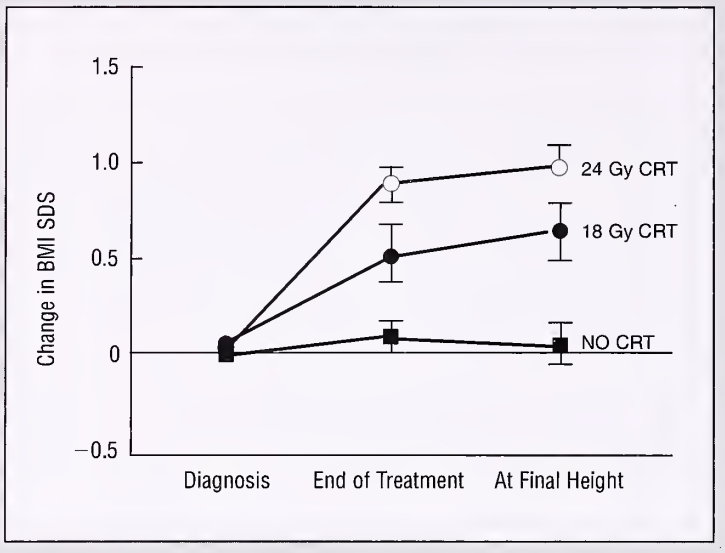
Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:
1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Figure 2



Change in body mass index (BMI)-standard deviation score (mean ± SEM) in survivors of acute lymphoblastic leukemia according to type of central nervous system prophylaxis. CRT, cranial irradiation.

Reprinted with permission from Sklar C, et al. [23]

one can justify on clinical grounds the time and expense involved in this diagnostic protocol. Hyperprolactinemia also can be observed following high-dose irradiation, particularly when more than 50 Gy are used to the hypothalamus. Associated with hyperprolactinemia, clinical symptoms such as secondary amenorrhea and galactorrhea occasionally occur.

Obesity is a well-established sequela of cancer therapy and is often observed in survivors of acute leukemia and various brain tumors. Sklar et al[21] and others suggest that in survivors of ALL a high incidence of obesity is seen but confined to those survivors who received cranial irradiation (Figure 2). Additional risk factors for *obesity* other than cranial irradiation include female gender and exposure to dexamethasone. The mechanisms underlying these propensities remain unsolved. It is unlikely, however, that the weight gain observed in the majority of individuals is of an endocrine basis. One possible explanation is that radiation damages centers within the brain that normally control eating behaviors and/or regulate body composition. Preliminary data suggest that cranial irradiation may even induce a state of relative leptin resistance.

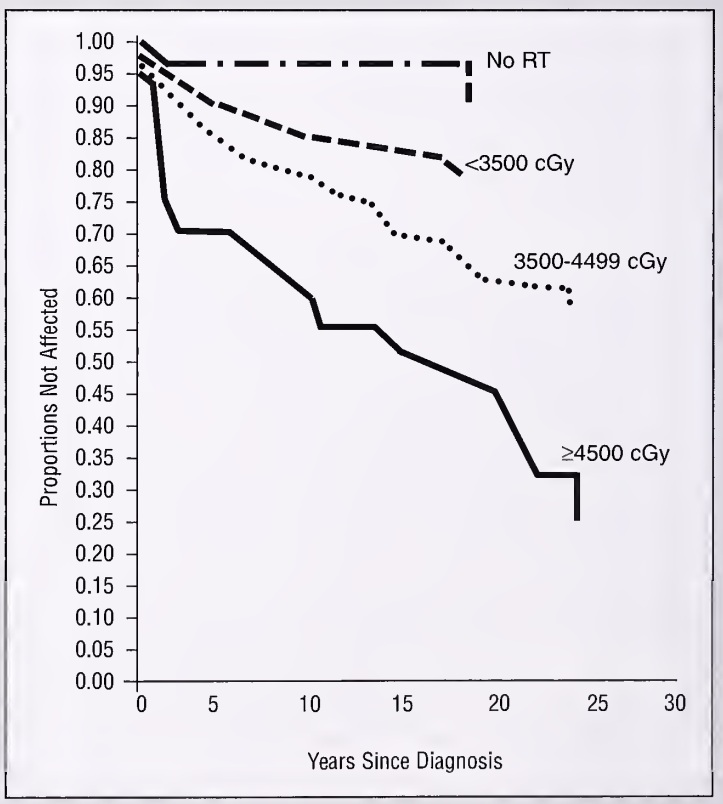
PRIMARY DISORDERS OF THE THYROID

Primary hypothyroidism is the most common thyroid disturbance that occurs in patients whose thyroid gland has been irradiated. Primary hypothyroidism generally results from direct damage to the gland following external beam radiation. Thus, it is often detected in survivors who have been treated with neck/mantle irradiation for Hodgkin's disease, cranial-irradiation for brain tumors, or total body irradiation for cytoreduction before bone marrow/stem cell transplantation.[8,22,23] Primary hypothyroidism also has been described in individuals treated with a radiolabeled monoclonal antibody such as I^{131}-MIBG for neuroblastoma.

As in other dysfunctions following chemotherapy or radiation therapy, the dysfunction is determined primarily by the total dose to the thyroid and by the duration of follow-up. In a recent study of 1791 young adult survivors of Hodgkin's disease, a cumulative incidence of hypothyroidism of 28% was observed.[23] Moreover, the actuarial risk of developing an underactive thyroid 20 years after treatment was 50% for survivors who had received thyroid irradiation with doses ≥45 Gy (Figure 3). Additional risk factors for developing hypothyroidism included female gender and/or being older than 15 years of age at the time of diagnosis. Of great clinical importance, new cases have been observed more than 25 years following diagnosis and treatment of Hodgkin's disease. Consequently all patients undergoing radiation therapy in the thyroid area deserve many years of annual observation.

Hyperthyroidism, while far less prevalent than hypothyroidism, does develop at an increased rate in certain subsets of childhood cancer survivors. A common setting is following external beam radiation to the neck region for Hodgkin's disease, where the chances of becoming hyperthyroid are 8 times greater than that observed in the general population.[23] The major risk factor for development of hyperthyroidism is irradiation of

Figure 3



Probability of developing an underactive thyroid after diagnosis of Hodgkin's disease. Patients are grouped according to dose of thyroid irradiation. RT, radiation therapy.

Reprinted with permission from Sklar C, et al.[23]

the thyroid involving doses >35 Gy. A second but less common cause of hyperthyroidism is the appearance of autoimmune thyroid disease following allogeneic bone marrow/stem cell transplant. The published data are most consistent with the hypothesis that the thyroid disorder is due to adoptive transfer of abnormal clones of T or B cells from donor to recipient.[8] Various types of autoimmune disease have been demonstrated to occur at increased frequency following bone marrow/stem cell transplants.

Thyroid neoplasms, both benign and malignant, do occur following irradiation of the thyroid gland. Children at greatest risk are those <5 years of age at the time of treatment and those treated with doses of radiation <20 Gy. Nonetheless, the risk of developing a thyroid neoplasm remains elevated following even relatively high-dose radiation therapy. Thyroid nodules are particularly common in females and often occur after a long latency period (>10 years). Recently, Sklar et al[23] reported that the risk of thyroid cancer was increased 18-fold in a large cohort of young adult survivors of Hodgkin's disease. The median dose of radiation to the thyroid was 35 Gy, with a range of 25 to 35 Gy. Fortunately, the vast majority of cancers noted after radiation therapy are well differentiated and have an excellent prognosis.

PRIMARY GONADAL DYSFUNCTION

Treatment-induced Leydig cell failure and/or dysfunction results from damage or loss of the machinery required for testosterone synthesis and release. Leydig cell failure and androgen insufficiency are relatively uncommon compared with damage to germ cells and infertility following cancer therapy. Chemotherapy-induced Leydig cell failure resulting in androgen insufficiency and requiring testosterone replacement therapy is quite rare.[24] As the majority of males undergo a normal puberty and most produce normal adult levels of testosterone, Leydig cell dysfunction is generally subclinical when it occurs. Subtle forms of Leydig cell dysfunction may be observed following chemotherapy protocols utilizing high doses of one of several alkylating agents.

External irradiation is more likely than chemotherapy to cause Leydig cell damage. The doses required are much higher than the doses needed to cause germ cell failure. The data obtained from individuals treated with radiation therapy for a variety of malignancies show that the likelihood of sustaining radiation-associated Leydig cell failure is *directly* related to the dose delivered and *inversely* related to age at treatment. Normal amounts of testosterone are produced by the majority of males who receive ≤20 Gy fractionated radiation to the testes.[24] Since raised concentrations of LH at baseline and following GnRH stimulation are found in many of these young men, one must assume that subclinical injury to the Leydig cells occurs even at these low levels of radiation exposure. The clinical importance of this phenomenon is unclear, but there are data to suggest that subtle forms of Leydig cell insufficiency may predispose to decreased bone density and changes in body composition over time.[25] A dose of >24 Gy fractionated irradiation as therapy for young males with testicular relapse of ALL is associated with a very high risk for Leydig cell dysfunction. One should anticipate that all boys who are prepubertal at the time that they receive 24 Gy testicular irradiation will also develop frank Leydig cell failure and require androgen replacement. Most but not all boys who are older and/or in early puberty at the time they are treated with 24 Gy will also ultimately need therapy with testosterone.

Table 1
Chemotherapeutic Agents Associated With Germ Cell Damage

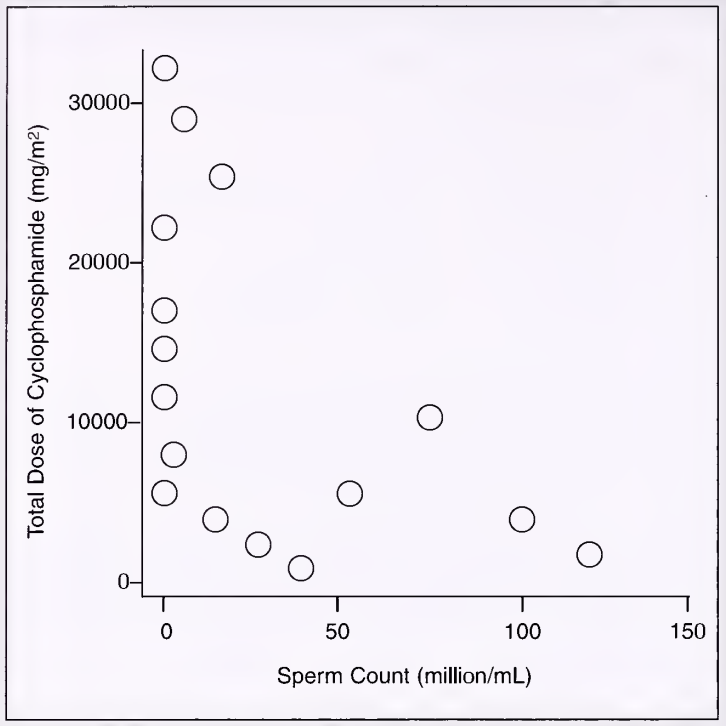
Alkylating agents	**Nitrosoureas**
Cyclophosphamide	BCNU (carmustine)
Ifosfamide	CCNU (lomustine)
Procarbazine	
Busulfan	**Cisplatin**
Melphalan	
Thiotepa	**Etoposide**

Treatment-induced germ cell failure in males occurs frequently, in contrast to what occurs in Leydig cells, which are resistant to damage from most chemotherapeutic agents and lower doses of radiation. The chemotherapeutic agents most commonly associated with impaired male fertility include the alkylating agents listed in Table 1. Importantly, the concept derived from earlier studies suggesting that the germ cells of younger males were less vulnerable to the toxic effects of chemotherapy compared with older boys and young adults has been called into question by recent studies.[26] Impaired fertility occurs in 40% to 60% of young adult male survivors of childhood cancer. A high probability of oligospermia azoospermia and infertility exists in those exposed to >20 g/m^2 of cyclophosphamide. In contrast, many individuals treated with a cumulative dose of 7.5 to 10 g/m^2 or less retain normal sperm production (Figure 4, page 42). [26,27]

Testicular irradiation in doses as low as 0.15 Gy has produced impaired sperm production. If the dose is under 1 to 2 Gy, recovery is generally common. At doses >2 to 3 Gy, recovery of sperm production is rare.[28]

Infertility resulting from radiation therapy or chemotherapy is often associated with reduced testicular volume, increased FSH concentrations, and reduced plasma concentrations of inhibin B. While there are good correlations overall between these markers and sperm counts in large groups of survivors, considerable overlap occurs between normal and abnormal individuals. *Many male survivors with documented azoospermia fail to manifest either a reduced testicular volume or an elevated level of FSH.* Thus, currently there is no substitute for sperm analysis to determine a male's current fertility status.[29]

Figure 4



Relation between total dose of cyclophosphamide (mg/m²) and sperm count.

Reprinted with permission from Relander T, et al.[27]

Ovarian failure results in disruption of and damage to both ovarian germ cells and the hormone-producing cells. This results from the structural and functional interdependence within the follicle between sex hormone-producing cells and oocytes.[24] This contrasts with testicular pathology, where despite the loss of germ cells following cytotoxic therapy, production of sex hormones is often preserved.

The ovaries of prepubertal females are relatively resistant to chemotherapy-induced damage compared with the ovaries of adults. Nonetheless, alkylating agents (Table 1, page 41) given at high doses can be toxic to the young ovary. Fortunately, the majority of prepubertal girls and adolescent females receiving standard combination chemotherapy will retain or recover ovarian function during the immediate posttreatment period.[24]

In young women treated with alkylating agents for acute leukemia, brain tumors, and Hodgkin's disease, increased plasma concentrations of FSH have been reported. Fortunately, normalization of FSH levels occurs in a majority, and only a minority experience irreversible ovarian failure. Recovery may not occur for many years following completion of therapy.[30] Even with FSH elevated at 5 years following completion of therapy, normalization and subsequent pregnancy have been reported. Some of these women, however, experience premature menopause when they reach their 20s and 30s.[31]

Females who receive high-dose myeloablative therapy such as busulfan, melphalan, and thiotepa with alkylating agents in the context of bone marrow transplantation are at high risk of developing ovarian failure.

Females receiving abdominal, pelvic, or spinal irradiation are at increased risk of ovarian failure, especially if both ovaries were within the treatment field. As is true for chemotherapy, damage from radiation therapy seems to be less severe in younger individuals than in older (adult) individuals. Thus, while radiation doses of 6 Gy may be sufficient to produce irreversible ovarian damage in women >40 years of age, doses in the range of 10 to 20 Gy are needed to induce permanent ovarian failure in the majority of females treated during childhood.[24,32]

SUMMARY

Our understanding of the endocrine consequences of cancer therapy has increased substantially over the past few years. Radiation therapy and chemotherapy are capable of causing damage that is often subtle; endocrine abnormalities may remain subclinical for many years. Physicians who follow children or adolescents who have been treated with chemotherapy and/or radiation therapy for cancer must encourage the patients to continue lifelong surveillance for potential endocrine disease.

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Cranial Irradiation and Central Hypothyroidism

Survivors of therapy for tumor infiltration of various sorts in the hypothalamic-pituitary area frequently have tropic hormone deficiencies. These deficiencies are known as central hormone deficiencies and produce "secondary" hormone deficiencies of the peripheral endocrine glands (thyroid, adrenal, gonads). Destruction of these peripheral glands directly results in primary thyroid, adrenal, and/or gonadal deficiency. Patients receiving irradiation of the head often end up with either primary or secondary thyroid deficiency—or both—because the thyroid and pituitary glands both receive irradiation as a result of the proximity of the thyroid gland to the hypothalamic-pituitary area. Rose reviews in this article the effects of cranial irradiation on regulatory cells of the pituitary gland. She then summarizes the characteristics of mild hypothyroidism (primary, central, and mixed) resulting from radiation therapy, discusses diagnostic methods, and recommends guidelines for the treatment of central hypothyroidism.

Rose's review is too extensive to abstract for *GROWTH, Genetics & Hormones,* but its thoroughness and importance must be brought to the attention of any physician working with children or adults who have received intracranial irradiation. Early in the article the effect of different types and doses of radiation upon various physiologic parameters is covered. The following section pertains to regulation of the thyroid axis and emphasizes the circadian pattern of thyrotropin secretion and how measurement of the normalcy or abnormalcy of this parameter is helpful in differentiating central, primary, and mixed hypothyroidism. The third section discusses primary hypothyroidism resulting from mantle irradiation for Hodgkin's disease, cranial irradiation for medulloblastoma, and total body irradiation in preparation for bone marrow transplant. Dr. Rose emphasizes that primary hypothyroidism is very frequently associated with secondary hypothyroidism in these instances. Central or secondary hypothyroidism then is considered; emphasis is placed on the frequent occurrence of free thyroxine (T_4) levels in the low normal range and on the absence of elevated thyrotropin levels. A blunted or absent nocturnal thyrotropin surge is a characteristic of central hypothyroidism, suggesting loss of the normal circadian variation in thyrotropin-releasing hormone (TRH) release. Mild hypothyroidism, both central and primary as well as mixed, is considered. Dr. Rose urges treatment for all patients with mild hypothyroidism, whether primary or secondary. "Even mild TSH (thyrotropin) rises might be a sign of possible thyroid dysfunction and should not be ignored. The opportunity to improve growth rate will be missed."

A subsequent section considers *mixed hypothyroidism,* which is a newly named syndrome consisting of central hypothyroidism associated with elevated thyrotropin. Secretory dynamics are abnormal.

A subsequent section deals with treatment of central hypothyroidism. One recommendation is that T_4 therapy in patients with central hypothyroidism should be adjusted to keep the free T_4 values at 1.4 to 1.6 ng/dL.

Dr. Rose observes that the cause of poor growth in childhood cancer survivors cannot always be identified. Although often caused by toxic effects of chemotherapy, radiation effects on bone growth centers, or GH deficiency, poor growth also can in many cases be caused by undiagnosed central hypothyroidism. Central hypothyroidism is much more common after radiation therapy for childhood cancer than has generally been recognized. Early identification and treatment of hypothyroidism can improve the quality of life and optimize the final adult height of these patients.

Rose SR. *Trends in Endocrinol & Metab* 2001;12(3):97-104.

Editor's comment: *The use of free T_4 screening and of confirmatory testing that combines the thyrotropin surge test with the TRH test should improve the sensitivity with which central hypothyroidism is diagnosed. The thyrotropin surge and TRH tests should be used to assess thyroid status in cancer survivors whose free T_4 value is in the lowest third of the normal range, whose basal thyrotropin concentration is normal, and whose growth rate is slowed. Other hypothalamic-pituitary axes should be evaluated concurrently as clinically indicated. Much improvement in diagnosing and treating primary, secondary, and tertiary hypothyroidism has occurred in the last 10 years. Dr. Rose's article is an excellent summary of these advances and how to apply them.*

Robert M. Blizzard, MD

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Final Height of Short Subjects of Low Birth Weight With and Without Growth Hormone Treatment

Zucchini et al report on their analyses of final heights in 2 groups of short children who were below the 10th percentile for *weight*. The 49 subjects presented at approximately 10 to 11 years of age. Thirty-five were below the 3rd percentile for height and 15 were between the 3rd and 10th percentiles for height. The latter were growing <3 cm/y. All had predicted heights lower than target heights, which were defined as sex-corrected midparental height (father's + mother's height) ÷2 +6.5 cm for males and −6.5 cm for females, expressed in SDS units. Each subject underwent 2 tests for GH release, arginine and levodopa stimulation. Those (29) with a peak of <8 µg/L were classified as GH deficient (GHD) and treated with GH 20 U (~7 mg/m²/wk), which at the average weight per m² equals 28k. On the average, such a child has a height age of 8 years. Therefore, for this size child, administration of 0.25 mg/kg/wk of GH is slightly less than the usual dose of 0.3 mg/kg/wk given in the United States to GHD patients. Treatment ranged from 36 to 84 months, with a median of 55.7 months. Final height was determined when growth was less than 0.5 cm in the last 6 months of GH treatment or at a chronologic age (CA) greater than 16 years (females) or 18 years (males). All subjects went through puberty spontaneously and had completed pubertal development by the end of the study.

Table
Results in the 2 Groups of Subjects Studied

	Final Height	Target Height–Final Height	Cases With Final Height> Target Height
Untreated group (n=20)	−1.87 (0.21)	0.65 (0.20)	6/20 (30%)
Males (n=9)	−1.81 (0.31)	0.56 (0.30)	3/9 (33%)
Females (n=11)	−1.92 (0.30)	0.75*(−0.33 + 1.35)	3/11 (27%)
Treated group (n=9)	−1.78 (0.18)	0.61 (0.18)	7/29 (24%)
Males (n=16)	−1.77 (0.25)	0.63 (0.27)	4/16 (25%)
Females (n=13)	−1.80 (0.25)	0.83*(0.07 + 1.20)	3/13 (23%)

In the first 2 columns data are expressed in SDS as mean (SEM) or median* (interquartile range).

Reprinted with permission from Zucchini S, et al. *Arch Dis Child* 2001;84:340-343.

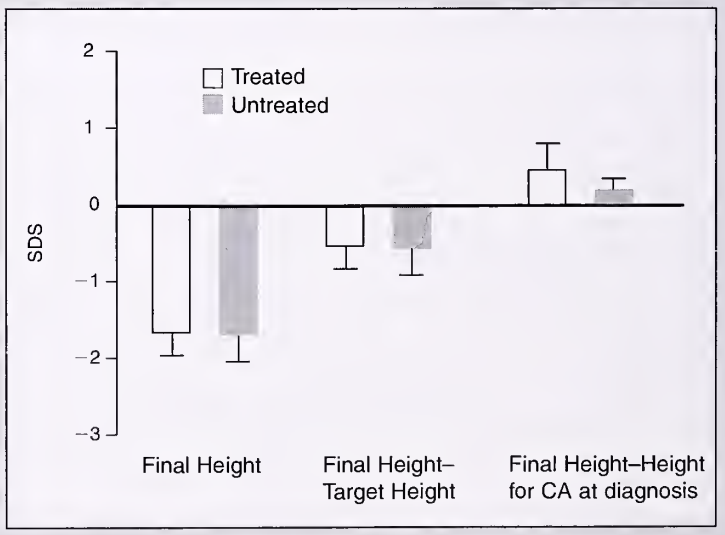
Both groups were similar at the initiation of the study with regard to birth weight, CA, height for CA SDS, height for bone age (BA) SDS, predicted height SDS, and target height SDS. Unfortunately, there was no statistical difference between the 2 groups when final height was measured (Table). Final height was significantly lower than the target height in both groups, and fewer than one third of the subjects reached a final height above their target height. In the untreated group, the height for CA SDS at diagnosis was the largest contributor to the variance in final height, followed by CA at diagnosis. In the treated group, height for BA SDS was followed by height for CA SDS and then CA at diagnosis, in respect to contributing variance. The Figure below graphically displays the lack of effect on the statistics of the 2 groups.

The authors state that their study confirms a negative prognosis for adult height when postnatal short stature persists, and that short subjects with low birth size will not reach their target height regardless of treatment with GH. They compare their data to that of Coutant and colleagues (*J Clin Endocrinol Metab* 1998;83:1070), who used lower doses of GH (0.4 U or 0.13 mg/kg/wk/ m² for a child) in 70 intrauterine growth retarded (IUGR) children with alleged GHD (not supported with retesting as adults) and compared the resultant data with an untreated comparable group. Final heights were comparable in both groups. Treatment was associated with a suggestive height gain of about 3.4 cm. The authors concluded that GH at this dosage level in IUGR GHD-classified patients had a limited effect on the final height of short children born with IUGR. Only those children starting treatment from a greater height for CA, and BA, and those with shorter parents had a chance of becoming taller than their parents in this study.

Zucchini S, et al. *Arch Dis Child* 2001;84:340-343.

Editor's comment: *This interesting study confirms the clinical observations of many pediatric endocrinologists, ie, most*

Figure
Lack of Effect in the 2 Groups of Subjects Studied



Final height, final height–target height, and final height–height for CA at diagnosis in untreated and treated sujects.

Reprinted with permission from Zucchini S, et al. *Arch Dis Child* 2001;84:340-343.

children with IUGR do not grow well even when given GH. Although the current study was in part retrospective, in that the physicians did not examine the children at the time of birth, they were able to determine that none of the children had any syndromes associated with short stature. The strength of the study is that it is one of the first to examine final height in these children. However, before placing undue credence on the findings, it should be noted that the children in both groups were relatively old (~10.8 years) when they presented for evaluation, and, therefore, there was little time for GH treatment prior to the onset of puberty. Despite these drawbacks, this relatively large study with final heights provides important information for physicians trying to determine whether to treat similar children with GH.

William L. Clarke, MD

Second editor's comment: *Although substantial data indicate that administration of rhGH increases growth rate and height in short children and adolescents with IUGR selected on the basis of low birth weight or short birth length,[1] there are few data concerning the adult height of such subjects.[2] (The term "near adult" height rather than "final" height is preferred by this commentator as the latter conjures up a vision of the ultimate "finality.") Sas et al[3] note that administration of rhGH over 6 years to children with IUGR (birth length <3rd percentile) has no apparent deleterious effect upon glucose disposal, although fasting insulin and glucose concentrations, the insulin:glucose ratio, and the insulin secretory response to oral glucose increased. Given the increasing evidence that impaired insulin sensitivity in subjects with IUGR untreated with rhGH may have possible long-term adverse consequences (hypertension, hypertriglyceridemia, ischemic heart disease, impaired glucose tolerance[4]), augmenting this potential problem with rhGH is an area of concern. Lastly, present data demonstrate once more (as if further evidence is necessary) the fallibility of provocative tests and the arbitrariness of GH concentrations in the assessment of GH secretory status in the absence of known anatomic, infectious, radiation, or neoplastic insults to the hypothalamic-pituitary axis.*

Allen W. Root, MD

1. de Zegher F, et al. Growth hormone treatment of short children born small for gestational age: growth responses with continuous and discontinuous regimens over 6 years. *J Clin Endocrinol Metab* 2000;85:2816-2821.
2. Ranke MB, Lindberg A. Growth hormone treatment of short children born small for gestational age or with Silver-Russell syndrome: results from KIGS (Kabi International Growth Study), including the first report on final height. *Acta Paediatr* 1996;417(suppl):18-226.
3. Sas T, et al. Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. *Clin Endocrinol* 2001;54:243-251.
4. Botero D, Lifshitz F. Intrauterine growth retardation and long-term effects on growth. *Curr Opin Pediatr* 1999;11:340-347.

Short Stature in Noonan Syndrome: Response to Growth Hormone Therapy

Noonan syndrome is a common syndrome occurring in both males and females; prevalence is approximately 1:1000. The gene for Noonan syndrome is found on chromosome 12p. Eighty-three percent of affected children in one series had short stature. Birth weight is usually normal but growth falls off before puberty, which is delayed. Final height is often compromised; mean adult height for males is 162.5 cm and for females 152.7 cm. There is no evidence of GH deficiency. Cardiac anomalies are frequent.

Kirk et al report on change in height SDS of 66 patients (54 males, 12 females) with Noonan syndrome, of whom 10 were treated with GH for up to 6 years. Seventy-eight percent of the subjects had a cardiac malformation, and 67% of the males suffered from cryptorchidism. The assessment of anterior pituitary function in 55 patients demonstrated normal GH secretion in all. Children with Noonan syndrome in one series had a height SDS of −2.9 compared with the normal population. The mean age at initiation of treatment was 10.2 years (±3.3). Seven of the 66 were experiencing pubertal development. The mean dose of GH was 0.79 U/kg/wk. Therapy with GH induced a significant increase in linear growth the first year, with subsequent falloff by the 4th year so that there was pretreatment growth velocity from year 4 on. The height SDS increased from −2.9 at the start of therapy to −2.3 after 6 years. The final height data were available only for 10 patients who were treated to near final height. The mean final height was 147.2 cm in girls and 159.9 cm in boys. These results are not greater than the average height of girls and boys with Noonan syndrome who are not treated with GH.

The authors note that information on long-term therapy is often limited to small numbers of patients in other studies. The National Cooperative Growth Hormone Study in the United States has registered 150 patients treated with GH. The data in that study were similar to those in the study reported here. In the Kabi International Growth Study (KIGS) from Europe, there were 143 patients in the registry treated with GH with an increase in height SDS of 0.5 for boys and 1.1 for girls after 3 years of therapy.

The authors conclude that GH therapy for up to 6 years in a group of short patients with Noonan syndrome has been shown to increase height velocity and height SDS compared with both normal and Noonan children, although there is a waning of effect after 3 years. Only a minority of patients improved their height prediction by more than 5 cm even though treated for longer than 3 years. This is similar to the response to GH seen in patients with Turner syndrome. Further prospective studies are required to see whether GH has a long-term benefit in Noonan patients.

Kirk J, et al of the UK KIGS Executive Group on Behalf of the Participating Centers. *Arch Dis Child* 2001;84:440-443.

Editor's comments: *This study is important for the data it presents on long-term GH treatment of Noonan syndrome. A recent article by MacFarlane et al (*J Clin Endocrinol Metab *2001;86:1953) noted a waning of growth effect after 3 years of GH treatment. It is possible that the optimal dose of GH for Noonan syndrome has not yet been determined and that, as in the treatment of Turner syndrome, it is a greater dose (based on kilogram of body weight) than usually prescribed for children with idiopathic GH deficiency. Unfortunately, the studies to date do not show an extremely positive response for patients with Noonan syndrome.*

William L. Clarke, MD

A Comparison of hGH and IGF-I as Growth-Promoting Agents in Children

Messina et al report the near adult stature of 2 children with isolated GH deficiency type 1A due to partial or complete deletion of the gene complex encoding the human *GH* gene cluster on chromosome 17q22-q24. In the first patient, only the gene encoding *CS-B* was retained; she was treated with rhGH for 12 years and achieved a near adult stature greater than her target height (153 cm vs 149 cm). This patient developed only a low titer of rhGH antibodies with low binding capacity. In the second subject, only the *GH-N* gene was deleted; the patient responded well to the administration of rhGH for 4 years (0.6 to 4.6 years) without development of antibodies to rhGH (height SDS increased from −5.0 to −1.4), but then abruptly developed a high titer of rhGH antibodies with high binding capacity that severely restricted the linear growth response to further rhGH administration (7.3 cm between 4.6 to 8.6 years). This child then received recombinant human insulin-like growth factor 1 (rhIGF-1) (8.6 to 13.9 years; 40 to 120 μg/kg SC twice daily); height increased only 21.2 cm during rhIGF-1 administration and the achieved near/adult height was far less than target height (128.6 cm vs 153.6 cm).

Backeljauw et al describe the linear growth response to rhIGF-1 (80 to 120 μg/kg SC twice daily) in 5 children with loss-of-function mutations in the GH receptor (Laron syndrome) and 3 with deletion of the *GH* gene and acquired GH insensitivity due to development of high titers of antibodies to rhGH during treatment with this agent. The response to rhIGF-1 was similar in the 2 groups. Overall, the mean pretreatment height SDS was −5.6, (range, −3.4 to −7.0); after 6.5 to 7.4 years of rhIGF-1 administration, mean height SDS was −4.2 (range, −1.5 to −6.6), and only 1 child had achieved a height SDS greater than −2.0. The mean pretreatment growth rate was 4.0 cm/y and increased to 9.3 and 6.2 cm/y during the first 2 years of rhIGF-1 administration but slowed thereafter. Head circumference, weight and fat mass, spleen and kidney size, nasopharyngeal lymphoid tissue, facial soft tissues, and bone mineral density increased during treatment with rhIGF-1.

The authors of both articles concluded that the linear growth response to rhIGF-1 of GH-insensitive subjects is far less than that of GH-deficient patients to rhGH. They attribute the variation in response, in part, to the different effects of GH and IGF-1 on early chondrocyte differentiation and later clonal proliferation, respectively.

Messina MF, et al. Final height in isolated GH deficiency type 1A: effects of 5-year treatment with IGF-I. *Eur J Endocrinol* 2001;144:379-383.

Backeljauw PF, Underwood LE, and the GHIS Collaborative Group. Therapy for 6.5-7.5 years with recombinant insulin-like growth factor I in children with growth hormone insensitivity syndrome: a clinical research center study. *J Clin Endocrinol Metab* 2001;86:1504-1510.

Editor's comment: *It was disappointing to learn that administration of rhIGF-1 did not restore normal linear growth in children with GH insensitivity. It is now apparent that the circulating concentration of IGF-1 is not as important a determinant of linear growth as is its tissue level. In mice without hepatic IGF-1 production, serum IGF-1 concentrations are low but linear growth is normal, suggesting that it is the local synthesis of IGF-1 that is critical for cartilage proliferation and bone growth. Since serum concentrations of "free" IGF-1 are normal in the animals without hepatic IGF-1 production, they might have accounted for the normal growth of these animals. However, the present studies in humans, in whom it is likely that during treatment "free" IGF-1 values were normal if not high (as IGF-binding protein-3 levels are low in these patients), suggest that it is not circulating but tissue IGF-1 values that are of greater importance for cartilage proliferation and linear growth. It will be of interest to examine the phenotype and response to therapy of the experimental mouse with dual knock-out of the genes encoding the GH receptor and hepatic IGF-1 synthesis.*

Allen W. Root, MD

Butler AA, LeRoith D. Minireview: tissue-specific *versus* generalized gene targeting of the *igf1* and *igf1r* genes and their roles in insulin-like growth factor physiology. *Endocrinology* 2001;142:1685-1688.

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FGF23, PEX and Hypophosphatemic Rickets

Hypophosphatemia occurs in a number of clinical settings, perhaps most apparent to pediatric endocrinologists and medical geneticists in X-linked and in the less common autosomal dominant forms of hypophosphatemic rickets, XLHR and ADHR, respectively. Both conditions are characterized by short stature, bow legs, hypophosphatemia, and radiographic changes of rickets and osteomalacia. A picture that explains the pathogenesis of these 2 inherited disorders and relates them to tumor-induced osteomalacia is beginning to emerge, and it involves an unlikely candidate, a relatively new member of the fibroblast growth factor (FGF) family, FGF23.

The recent story begins in 1995 with the identification by the HYP Consortium of mutations in a gene that maps to chromosome Xp22.1 in patients with XLHR.[1] The gene, which encodes a protein whose amino acid sequence suggests it is a neutral endopeptidase, was called *PEX* for "phosphate regulating gene with homologies to endopeptidases on the X chromosome." However, the substrates for PEX were not known. A number of mutations have subsequently been found that predict loss of function for the putative enzyme.[2]

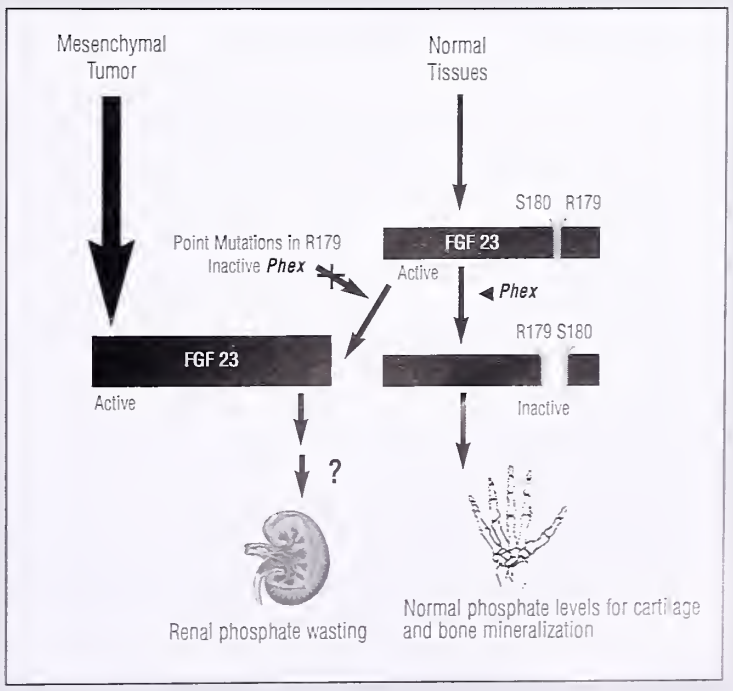
The next chapter occurred in late 2000 with the positional cloning of FGF23 as the gene that harbors mutations responsible for ADHR.[3] Of note was that the mutations in 4 families studied mapped to 1 of 2 closely spaced arginine residues at positions 176 or 179 of FGF23.

Most recently, Shimada et al have shown that FGF23 is produced abundantly in tumor-induced osteomalacia.[4] They first cloned a highly expressed cDNA from an osteomalacia-inducing tumor, showing that it encoded FGF23. Next, they demonstrated that injection of FGF23 into mice reduced serum phosphate levels within 12 hours. They then showed that transplantation of CHO cells expressing and secreting FGF23 into nude mice led to hypophosphatemia; increased phosphate renal clearance; high alkaline phosphatase and inappropriately low 1,25-dihydroxy-vitamin D levels in association with bone deformities; osteomalacia; and widening of the growth plate typical of rickets. Shimada et al were unable to demonstrate direct effects of FGF23 on phosphate transport in renal epithelial cells (OK cells) in culture, raising the possibility that FGF23 acts indirectly on renal phosphate transport. However, in an independent study, Bowe et al documented that FGF23 does block phosphate resorption in this cell culture model of renal proximal tubule epithelia.[5]

As this story evolved, the idea emerged that FGF23 is a substrate for PEX and that loss of PEX function in XLHR leads to an accumulation of FGF23 in serum and in kidney tissues, where it blocks renal phosphate resorption. Supporting this possibility is that the arginine residues (Arg176 and Arg179) that are mutated in all 4 families with ADHR are part of a consensus recognition sequence for endopeptidases, such as PEX. Indeed, Bowe et al have now confirmed that FGF23 is a substrate for PEX cleavage and that FGF23 harboring the Arg179Gln missense ADHR mutation is not cleaved in an in vitro assay.[5]

1. HYP Consortium. *Nat Genet* 1995;11:130-136.
2. White KE, et al. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet* 2000;26:345-348.
3. Sabbagh Y, Jones AO, Tenenhouse HS. *Hum Mutat* 2000;16:1-6.
4. Shimada T, et al. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Nat Acad Sci USA* 2001;98:6500-6505.
5. Bowe AE, et al. FGF-23 inhibits renal tubular phosphate transport and is a PEX substrate. *Biochem Biophys Res Commun* 2001;284:977-981.

Figure
Proposed Pathogenesis of Renal Phosphate Wasting



Mesenchymal tumors produce renal phosphate wasting by overproduction of FGF23 levels can also be increased by mutations in *Phex*, a protease that cleaves and inactivates the molecule, or by mutations at key arginine residues that render FGF23 resistant to cleavage by *Phex*. FGF23 excess causes phosphate wasting either directly or by inducing another phosphaturic factor.

Editor's comment: *These articles document that FGF23 is an important modulator of phosphate homeostasis and that this process is regulated at least in part by PEX through degradation of the growth factor. They further demonstrate that FGF23 levels and resulting phosphate homeostasis can be altered through several mechanisms, including excess production by tumors and by slowed degradation either because the enzyme that normally cleaves FGF23 is ineffective due to mutation or because the growth factor itself is mutated so that it is resistant to degradation. This concept is discussed in depth by Strewler and depicted in the Figure on page 47.*

William A. Horton, MD

Strewler GJ. FGF23, hypophosphatemia, and rickets: has phosphatonin been found? *Proc Nat Acad Sci USA* 2001;98:5945-5946.

Second editor's comment: *This work illustrates the treasure trove of genetic data already available from the Human Genome Project waiting to be mined for relevance to human physiology and pathophysiology. PEX is expressed by osteoblasts, and it has been hypothesized that "phosphatonin" also may be synthesized by these cells.[1] In normal mouse embryos, the murine homologue Fgf23 maps to chromosome 6. The present investigators were unable to demonstrate expression of Fgf23 in the tibiae of embryonic mice, perhaps suggesting that FGF23 is not "phosphatonin." Tumors that secrete a phosphate-wasting product leading to rickets or osteomalacia have been demonstrated by the same group to express FGF23 mRNA and to synthesize FGF23 protein.[2] However, it has not as yet been shown that FGF23 has phosphaturic activity or acts upon yet another molecule, the still elusive "phosphatonin."[3]*

Allen W. Root, MD

1. Ecarot B, Desbarats M. 1,25-$(OH)_2D_3$ down-regulates expression of PHEX, a marker of the mature osteoblast. *Endocrinology* 1999;140:1192-1199.
2. White KE, et al. The autosomal dominant hypophosphataemic rickets (ADHR) gene is a secreted polypeptide overexpressed by tumors that cause phosphate wasting. *J Clin Endocrinol Metab* 2001;86:497-500.
3. Quarles LD, Drezner MK. Pathophysiology of X-linked hypophosphatemia, tumor-induced osteomalacia, and autosomal dominant hypophosphatemia: a perPHEXing problem. *J Clin Endocrinol Metab* 2001;86:494-496. Editorial.

Ethical Issues With Genetic Testing in Pediatrics

Advances in genetic research and emerging genetic technology are enabling testing and screening to be implemented before a full understanding of the ramifications has been developed. Clearly, new developments in genetics should be made available if they promote the best interest of the patient, in this case the child. The Committee on Bioethics of the American Academy of Pediatrics (AAP) reviewed the issues involved in genetic testing and put forward principles that should be considered before genetic testing is provided to an infant, child, or adolescent. Their report cites the Institute of Medicine's report of 1994 assessing genetic risks, implications for health, and social policy in which 3 principles were described for the introduction of new genetic tests: (1) Identification of the genetic condition must provide a clear benefit to the child; (2) a system must be in place to confirm the diagnosis; and (3) treatment and follow-up must be available for the affected individuals.

Although genetic research offers great promise for the improvement of health, the use of genetic testing must be considered carefully and only introduced with full and appropriate informed consent for the parents who provide consent for the child to have testing. There are several critical reasons for this. Genetic testing is different than other types of laboratory testing since the information obtained is familial and thus has implications for other family members. The risks of genetic testing may not be obvious but include psychosocial risks such as guilt, anxiety, and impaired self-esteem, social risks such as stigma, and financial risks involving insurance and employment. Genetic information may have limited predictive power since diseases are very complex and there are multiple environmental and genetic variables. Genetic conditions may be difficult to treat or prevent without additional research. The positive aspects of making a diagnosis should be demonstrated before screening tests are implemented.

The AAP committee report points out that there are insufficient numbers of genetic professionals (genetic counselors and clinical geneticists) to have primary responsibility for managing the use of genetic testing, and, thus, primary care physicians must become knowledgeable about both the limitations and the positive aspects of genetic screening in children. It is particularly important to provide or refer children for counseling and testing only when it is in the best interest of the child and when testing and counseling can be provided without anticipated harm to the child.

The committee report is broken down into newborn screening, carrier screening, and predictive testing for late-onset disorders. Under newborn screening, it is reiterated that the purpose of newborn screening for genetic disorders is to limit the morbidity and mortality attributable to these inherited diseases. The report indicates that mandatory and voluntary screening should be distinguished. It strongly suggests that informed consent and voluntary screening occur rather than mandatory screening. The informed consent improves the efficiency of response to positive results and incorporates outcomes research if parents are already involved in making the decision to screen. Newborn screening protocols for phenylketonuria and hypothyroidism have been the model for early diagnosis, leading to improved treatable outcomes; however, the evaluation of the consequence of informed refusal is not yet available.

Screening programs to detect carriers are associated with significant concerns about the possibility for communities to misunderstand the carrier state, leading to stigma and discrimination against the identified carrier, as well as the possibility of adverse psychological reactions. Nevertheless, carrier testing for pregnant adolescents or adolescents who plan pregnancies may well be appropriate.

Predictive testing for late-onset disorders is as yet poorly understood and in general should be delayed until an autonomous decision by the individual to have this type of

predictive testing can be made. Reduction in morbidity or mortality as a result of genetic testing for late-onset disorders has not yet been demonstrated, and the risk of adverse psychological response and discrimination by insurers and employers appear to be real concerns. Further, the complexities of genetic testing for complex disorders have not been worked out.

In summary, the AAP Committee on Bioethics points out that pediatricians must be well informed about these issues and understand that there are both positive and negative aspects of genetic screening that are part of proper informed consent. Furthermore, potential harm does exist in screening programs, and testing should be deferred until adulthood unless there would be significant benefit to the child to undergo genetic testing.

Committee on Bioethics. *Pediatrics* 2001;107:1451-1455.

Editor's comment: *The AAP report on genetic testing should be required reading for pediatricians since there are pitfalls to all genetic testing. These must be understood by both the pediatrician and the person giving permission for testing of a child before testing is undertaken. Therefore, search out the complete article.*

Judith G. Hall, OC, MD

Development of Renal Cell Carcinoma in Living Donor Kidney Grafts (in Association With hGH Administration)

Tyden et al report 2 cases of young boys (~4 years of age) who received kidney transplants from their fathers. De novo development of carcinoma was diagnosed by biopsy 9 and 11 years after transplant. One patient received a new transplant and the other received dialysis therapy. Progressive cyst formation was observed in each kidney for many years before carcinoma was diagnosed. The kidneys remaining in the 2 fathers did not develop cyst formation. The boys received human growth hormone (hGH) for a total of 7 years and approximately 5 years. For the latter, administration was intermittent.

The authors state that although renal cell carcinomas have developed previously in kidney allografts (cadaver source), it is not known whether in those reported cases the carcinomas were de novo or whether they had been present at transplantation. The authors, however, state that these are the first de novo cases reported in living donor transplants. The authors conclude that it is possible that hGH stimulates the growth of renal cell carcinoma, or perhaps induces the development of such carcinoma more quickly, in acquired disease of the kidney transplant. They also state that the findings emphasize the importance of annual ultrasonographic surveillance of renal grafts, especially in the pediatric population.

Tyden G, et al. *Transplantation* 2000;11:1650-1656.

Editor's comment: *Regardless of whether coincident with, or attributable to, hGH administration, the fact that renal cell carcinoma occurred in these 2 kidney recipients who were receiving hGH deserves significant attention. All transplanted patients should be followed closely for the possible development of renal carcinoma. Development of cysts should prompt suspicion that carcinoma might develop. The development of solid tumors superimposed on the cystic kidney should be reason for immediate surgery. The development of cysts in patients receiving hGH, in my opinion, should prompt discontinuation of hGH. Fortunately, the time intervals appear to be lengthy before renal cell carcinoma develops after transplant. The possibility that hGH might be an inductive agent for renal carcinoma, again in my opinion, should be discussed with the parents, and with the child if he/she is the age of consent, before hGH is administered. hGH should be given under the auspices of a research protocol.*

Robert M. Blizzard, MD

Growth Hormone Deficiency (GHD) Caused by Pituitary Stalk Interruption in Fanconi's Anemia

Fanconi's anemia can be associated with growth retardation. The authors describe the presence of isolated growth hormone deficiency (GHD) or GHD associated with thyrotropin deficiency in the pituitary stalk interruption syndrome, which was demonstrated by magnetic resonance imaging (MRI) in 5 patients with Fanconi's anemia. GH treatment produced catch-up growth in all cases. The authors concluded that the combination of these findings suggests a common genetic origin.

Dupuis-Girod S, et al. *J Pediatr* 2001;138:129-133.

Editor's comment: *Fanconi's anemia is a rare autosomal recessive disease of variable penetrance that arises from an abnormal processing of DNA. The first of the genes responsible for this syndrome was identified in this decade (*Nature *1992;358(6385):434). Fanconi's anemia patients may present with multiple congenital abnormalities, including bone marrow failure, and increased susceptibility to cancer. They have a 15,000 times greater risk of developing acute myelogenous leukemia (*Blood *1994;84:1650-1655).*

*It has long been recognized that growth retardation with normal or decreased GH response to pharmacologic stimuli may be present in this disease. The International Fanconi's Anemia registry reported that short stature is a common finding in these patients (mean, 22.37 SDS) with an 81% prevalence of endocrinopathy. Forty-four percent of the tabulated patients had a subnormal response to GH stimulants; 100% had an abnormal response to GH profile (*Pediatrics *2001;107:744-754). Dupuis-Girod et al in the present paper*

*demonstrated that Fanconi's anemia is frequently associated with GHD and pituitary stalk interruption syndrome. The demonstration by MRI of the latter abnormality is a new finding, which had not been documented in the past in such patients. The pathogenesis of pituitary stalk interruption syndrome is unknown. It could be related to injury at birth or perhaps to the same deletions in the genes that lead to Fanconi's anemia. It is interesting to note that patients with Fanconi syndrome might not always have the severe type of GHD. Pituitary stalk interruption probably needs to be considered only in patients with Fanconi's anemia who are severely growth retarded and in whom treatment with GH will induce catch-up growth. However, it should be kept in mind that patients with chromosomal abnormalities, including patients with Fanconi's anemia, in particular are at a higher risk for malignancies when treated with GH. Therefore, the question has been raised about the dilemma of initiating a treatment that may improve growth but also might increase the risk for cancer. Although the incidence of leukemia in GH-treated patients without predisposing risk factors is believed not to be different from that of the general population (*J Clin Endocrinol Metab *1996;81[693]:1692-1696 and 1704-1710), in patients with Fanconi's anemia this complication might ensue (*Lancet *1994;343:1576).*

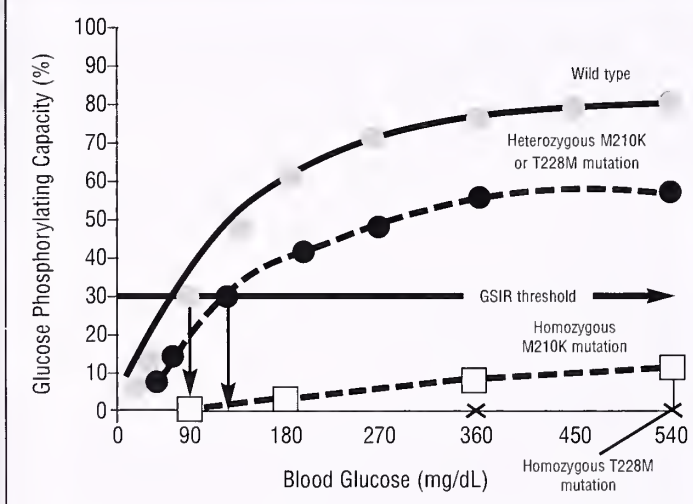
Fima Lifshitz, MD

Neonatal Diabetes Mellitus Due to Complete Glucokinase Deficiency

Diabetes mellitus is a heterogeneous disorder. Neonatal diabetes, defined as insulin-requiring hyperglycemia occurring within the first month of life, is a rare form of diabetes but also is heterogeneous. Transient or permanent neonatal diabetes can occur. Recently, it has been recognized that transient neonatal diabetes is often associated with abnormalities of chromosome 6, including imprinting abnormalities. Mutations of insulin promoter factor 1, resulting in pancreatic agenesis, are seen in permanent neonatal diabetes.

This report describes 2 patients with permanent neonatal diabetes due to complete glucokinase deficiency, the result of identified mutations in the glucokinase gene. The affected individuals had poor fetal growth and intrauterine growth retardation, and required insulin in the first days of life. Interestingly, diabetes of many forms was seen within the family among the carriers (heterozygotes) of the gene defects. Among the carriers (heterozygote), maturity-onset diabetes, diabetes of the young, type 1 diabetes, and type 2 diabetes were all observed. One affected infant also had total situs inversus, which was not seen in any other family members.

Figure



Variable	Wild Type	M210K Mutation	T228M Mutation
Turnover rate (sec^{-1})	52.4 ± 5.7	16.7 ± 1.6	0.004 ± 0.0004
Glucose $S_{0.5}$ (mmol/L)	7.7 ± 0.1	38.7 ±1.9	5.5 ± 1.5
Hill coefficient	1.66 ± 0.00	1.63 ± 0.04	0.71 ± 0.07
ATP_{km} (mmol/L)	0.35 ± 0.01	1.38 ± 0.11	0.62 ± 0.16

Comparison of the modeled functional properties of wild-type glucokinase, glucokinase with the M210K mutation, and glucokinase with the T228M mutation in the homozygous and heterozygous state. GSIR, glucose-stimulated insulin release.

Reprinted with permission from Njolstad PR, et al. *N Engl J Med* 2001; 344:1588-1592.

Glucokinase mutations are relatively common in diabetes, and the homozygous state may actually be a common cause for neonatal diabetes. Glucokinase plays a key role in the regulation of insulin secretion in humans. Thus, the authors tested for mutations in other genes along the pathway, including hepatocyte nuclear factors 1 and 4, insulin promoter factor 1, NK-2 homeobox homologue 2, neurogenic differentiating factor 1-beta-cell, and E box transactivator 2. They found no abnormalities in any of those genes.

Interestingly, mouse models of glucokinase deficiency also have growth retardation and hypoglycemia at birth, but they also have hypertriglyceridemia, hepatic steatosis, and reduced stores of glycogen, which apparently are not seen with the human mutations.

Njølstad PR, et al. *N Engl J Med* 2001;344:1588-1592.

Editor's comment: *The variabilities seen in the families of these infants with neonatal pronate diabetes are quite remarkable, suggesting that heterozygotes have problems of many varieties. The authors worked out the kinetics of complex control of glucose metabolism and showed very nicely that the homozygous state simply does not produce enough enzyme to have a normal role, whereas the heterozygous state has variable levels and thus must interact with other factors to produce the various types of diabetes seen (Figure).*

Judith G. Hall, OC, MD

Stem Cells to Pancreatic Islet, Insulin Secreting Cells

Stem cells are receiving considerable attention because of the ethical issues they raise and the potential they offer for treatment of many human diseases. The recent report that stem cells can be cajoled to produce insulin may raise the debate to a new level.

The endocrine pancreas (islets of Langerhans) contains 4 cell types that secrete peptide hormones: insulin (β cells), glucagon (α cells), somatostatin (δ cells), and pancreatic polypeptide (PP cells). Because of the close association of these cells with neural cells in the pancreas and their similar embryonic origins, the National Institutes of Health group headed by McKay postulated that experimental strategies that induce embryonic stem cells (ES cells) to become neural cells could be used to induce ES cells to become pancreatic endocrine cells.

Using techniques previously developed, they first induced ES cells to differentiate as neural precursor cells. These cells expressed a marker gene, termed nestin. Their protocol then progressed through a series of steps that sequentially expanded and selected pancreatic endocrine progenitor cells, using various markers to identify these cells and their precursors. By the end of the protocol, which took approximately 3 weeks, they generated relatively large numbers of insulin-positive cells, which resided in clusters in close association with neurons. Confocal microscopy revealed that the insulin-positive cells were located in the centers of the clusters surrounded by neurons. Immunostaining revealed that glucagon, somatostatin, and pancreatic polypeptide also were produced by cells in the clusters that tended to surround the insulin-positive cells. Pancreatic exocrine markers were not detected. Thus, the ES cells generated multicellular structures that resembled pancreatic islets in vivo.

The investigators next performed clonal analysis to determine if the islet-like cells and the neurons developed from independent progenitor cells or from a common progenitor cell. The results suggested they arose from a common progenitor cell pool.

Experiments were next carried out to show that the islet-like cells release insulin in response to glucose in a dose-dependent manner with kinetics similar to those of pancreatic islet cells in culture. Quantitation revealed that the ES cell-derived cells contained about 1/50th the amount of insulin that normal islet cells contain. The researchers then examined the effect of several known agonists and antagonists of insulin secretion on insulin release. All of the agents tested produced appropriate responses, indicating that the machinery used to regulate insulin secretion by islet cells is present in the islet-like cells.

Finally, the authors tested the ability of the ES cell-derived clusters to survive and function in vivo by grafting the cell clusters subcutaneously into the shoulders of streptozocin-diabetic mice. When harvested later, the clusters were shown to vascularize and to remain insulin-reactive. Although grafted mice were able to maintain body weight and survive longer than sham-grafted controls, they were not able to sustain normal blood glucose levels, which the authors attributed to the relatively low levels of insulin per cell of the ES cell-derived islet-like cells.

The researchers concluded that engineering of ES cells to produce an abundant source of immunocompatible tissue for transplantation holds considerable promise as a future strategy for treating diabetes. In an accompanying commentary, Vogel points out that although others have reported promising results in transplanting pancreatic cells from cadavers into diabetic patients, the demand for cells is far greater than the current supply.

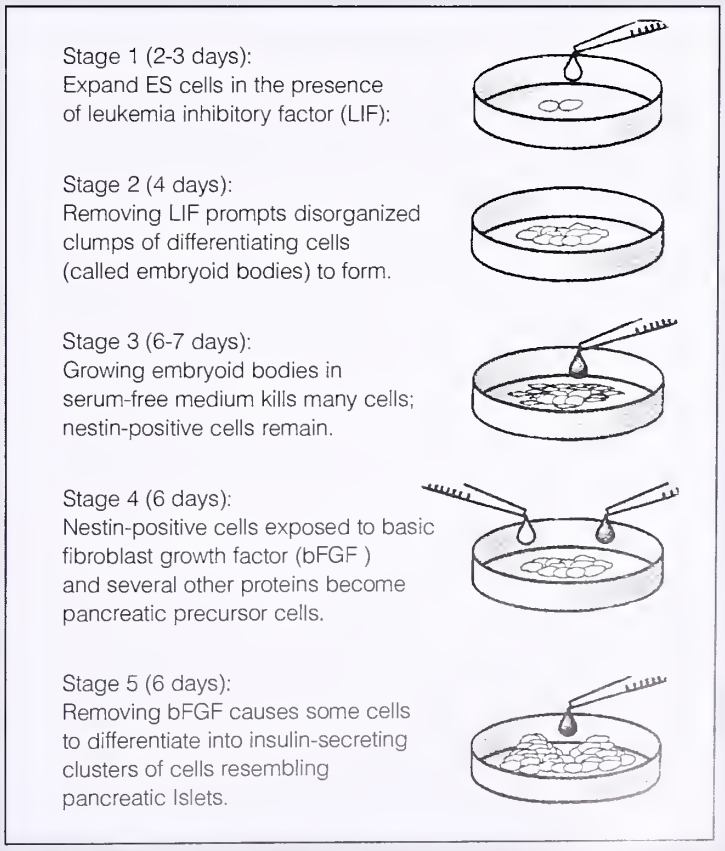
Lumelsky N, et al. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001;292:1389-1394.

Vogel G. Stem cells are coaxed to produce insulin. *Science* 2001;292:615-617.

Editor's comment: *As the authors acknowledge, these very promising results are still only preliminary. Nevertheless, they have caused excitement in the scientific community. It will be interesting to see if human ES cells behave like the mouse ES cells. If so, it will add considerable fuel to the debate over the use of ES cells to treat human disease.*

William A. Horton, MD

Figure
Turning Mouse Embryonic Stem (ES) Cells Into Insulin-Secreting "Islet Clusters"



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Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of this issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Chemotherapy, as suggested by the data, suppresses growth
   a. through alterations of the GH-IGF-1 system
   b. by suppressing osteoblast and osteoclast activity
   c. neither
   d. both

2. The ultimate impact on final height of direct external beam radiation depends on which of the following:
   a. the dose of radiation therapy
   b. the volume irradiated
   c. the race of the subject
   d. the gender of the subject
   e. the age of the subject

3. Endocrine complications as a result of irradiation usually occur in the first 5 years following treatment.
   a. True
   b. False

4. External irradiation of the testis is more likely than chemotherapy to cause Leydig cell damage.
   a. True
   b. False

5. A normal testosterone level following external irradiation is indicative that Leydig cells are not compromised.
   a. True
   b. False

6. Reduced testicular volume and elevated levels of follicle-stimulating hormone (FSH) following irradiation are reliable indicators that azoospermia is present. Azoospermia also occurs frequently in males receiving external beam irradiation whose testicular size and levels of FSH are normal.
   a. True
   b. False

Answer Key
1.d, 2.abe, 3.b, 4.a, 5.b, 6.a

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